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THE BOTANICAL REVIEW

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COLONIZATION OF ARTIFICIAL BARE AREAS BY MICROORGANISMS

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INTRODUCTION

A large number of microorganisms exist in nature in habitats in which they can be studied by any of several methods. The simplest methods are those in which samples of the habitat, such as water, soil or bottom materials from bodies of water, are brought to the laboratory and observed through special counting cells, plated in special agar or gelatin media, or selectively cultured with special nutrient substances. A more complicated procedure is to plant special nutrient " bait " in the habitat and wait for the microorganisms to become attached to the bait material, after which it is brought to the laboratory for study. Another type of technique is the exposure of smooth or relatively smooth objects, usually transparent, which after suitable exposure in the chosen environment are returned to the laboratory. Here the object is studied by direct observation or by more specialized techniques, with the hope that something may be learned concerning the microorganisms present, their speciation, abundance and relationship to each other as members of a community.

Since the early twentieth century, studies of the environments in which microorganisms grow have developed terms to describe or label the habitat. As knowledge of details increased, echelon upon echelon of micro-habitat types within major habitat types were postulated. Students in different areas developed techniques independently or improved on the technique of others, sometimes crediting the person who innovated the idea, sometimes not.

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In an effort to learn something of the nature of the fungal organisms which occur on trickling filters in sewage treatment plants, the writer borrowed, from a paper published in England (67), a technique which appears to have some use. The following review was developed partly to determine the extent to which similar techniques had been used in other studies of microorganisms and partly to search for an appropriate term to describe the community of organisms under study.

TERMINOLOGY

In aquatic environments there is a distinct group of organisms, including bacteria, fungi, algae, protozoans and even higher forms of animal and plant life, which grow attached to stones and other objects projecting from the bottom of a body of water as well as upon and around stems, leaves, shells and other portions of companion organisms. By many authorities the attaching organisms are considered part of the benthos (organisms growing on the bottom of a body of water), in contrast to the plankton, or plancton (organisms floating or swimming free in a body of water). As long as one divides aquatic microorganisms into only two groups, plankton and benthos, this classification is satisfactory. However, because of variations in habitat, habitat requirements, mode of life, type of adaptation to the environment, and other factors, it has often been found necessary to divide these two primary classes of aquatic life into a number of categories. Since the present discussion is concerned with only one phase of the benthos, the other phases of this class and the plankton will not be discussed further.

With the development of special techniques for the study of aquatic organisms of certain special habitats, there arose the need for terms to describe the organisms, their mode of life and the particular association (either plant or animal) or biocoenose (both plant and animal) in which these organisms were found. Several words have been used to describe organisms that are not able to move about in the water. Algologists say that algae are "attached" to the substrata by means of rhizoids, holdfasts or other devices. Zoologists say that such organisms are "sessile-attached". Glossaries and dictionaries give two meanings for the word "sessile": (*a*) attached directly by the base, not raised upon a stalk or peduncle; (*b*) permanently attached, not free to move

about. The first of these is a botanical definition, the second a zoological one. Thus in marine zoology an organism permanently attached to the substratum (bottom of the sea) is sessile-attached, while organisms associated with it, such as those more or less loosely attached to the stem, leaf or shell, and those minute organisms swimming or crawling over the primary organism in search of food, are merely sessile. This extended definition of " sessile " is found in the literature largely by implication rather than as direct expression. The term " sessile " is also applied to organisms which become attached to artificially exposed materials used for study of organisms in aquatic habitats, including any form of life so obtained, especially bacteria.

Sessile organisms may be minute single cells, stalkless masses of cells, stalked protozoans or other animals, algae and even strands of algae, or other organisms lying more or less impassively on the bottom of bodies of water. The sessile-attached organisms are also referred to as " sedentary ", but these, as associated with the " fouling " of piers, ship bottoms and other structures, are usually macro- instead of microorganisms. Non-motile planktonic forms are also considered sedentary.

It was reported (83) that the first use of the term " Aufwuchs " for organisms attached to but not penetrating a substratum was made in 1905 (62). In 1920 (73) the term was limited to include only those organisms occurring on living substrata. The term originated in the German literature and was used in both senses (50) until Ruttner (61) gave it specific meaning in description of aquatic life. This author divided the benthos into two biocoenoses: Aufwuchs and Ooze: " By the term Aufwuchs we mean all those organisms that are firmly attached to a substratum but do not penetrate into it ". " Aufwuchs comprises all attached organisms, except the macrophytes, including such forms as Bryozoa and sponges, which are usually considered as benthos by American authors; also included are the various forms living free within the mat of sessile forms. This is a broader term than periphyton, including both aerial and aquatic forms ".

Again in Germany, following a search for a term to apply to the organisms collected on glass plates exposed in the canals around the port of Hamburg, the word " Bewuchs " (28) was chosen, but it was used in a different sense than the original use of the word

" Aufwuchs ". In other German literature (75) this term was used for growth on submerged dead supports, in contrast to Aufwuchs which referred to growth on submerged living supports.

Apparently the first use of the term " periphyton " (2) was made in 1924. This term was applied to organisms growing atached to artificial objects in water. Later (3) this term was extended to cover all aquatic organisms growing on submerged surfaces. According to this usage we have three distinct classes of aquatic microorganisms: plankton, periphyton, benthos. In the United States (33) " periphyton " was apparently first applied to bacteria obtained on slides exposed in bodies of water in Minnesota. These were called " periphytic bacteria ", while in Russia (34) these organisms were referred to as the " bacterial periphyton ". " Epiphyte " has been used to apply to plants or plant-like organisms growing on plants, whether in air or water. In this way we reach the definitions that a periphyte is an organism growing on a submerged object or plant, while an epiphyte is an organism growing on a plant in the air without obtaining food, water or minerals from the supporting plant.

The following definition has been given for periphyton (83): " By periphyton is meant that assemblage of organisms growing upon free surfaces of submerged objects in water and covering them with a slimy coat. It is that slippery brown or green layer usually found adhering to the surface of water plants, wood, stones or certain other objects immersed in water and may gradually develop from a few tiny gelatinous plants to culminate in a wooly, felted coat that may be slippery, or crusty with contained marl or sand ". It was felt that this was a preferable term for this group of organisms, but it was emphasized that the term does not include organisms considered as belonging to the benthos. A recent text on limnology (73) gives about the same definition, but the meaning of the term is broadened somewhat in a more recent text on ecology (53): " Periphyton—organisms (both plant and animal) attached or clinging to stems or leaves of rooted plants or other surfaces projecting above the bottom ". The latter book goes further and indicates that the term " Aufwuchs " (61) was possibly preferable.

In Michigan (52, 53) the term " seeding-on " was used apparently as the English equivalent of " Aufwuchs ", especially for or-

ganisms obtained by exposing glass slides and other bare areas in bodies of water. In the same sense the same worker used the term " attachment materials ".

In the study of bacteria (86) obtained by exposing glass slides in ocean water in California, these organisms were referred to as " attachment bacteria ". Other workers studying free-occurring bacteria in ocean waters obtained " attachment counts " of bacteria on glass slides. It was felt that this was more descriptive than the contemporary " periphytic bacteria ", since the bacteria were thought to be not passively stuck but actively attached. At about the same time, in studies on the California coast (15, 16), the long-standing terms " sedentary organisms " and " fouling organisms " were used for those macroorganisms which foul ship bottoms, pilings of piers and other marine structures, thus causing loss or inconvenience to man.

Use of the term " littoral " for the sandy margins of lakes was considered confusing (51), since this term is commonly applied to sea shore areas. To resolve this confusion, sandy shores of lakes are termed " psammon ". This term is extended in certain areas: the " hydropsammon " is the littoral zone of a lake, or the inner beach; the " hygropsammon " is the eulittoral zone, or middle beach; while the " eupsammon " is the supralittoral zone or outer beach. The first is always covered with water, the last is never covered with water, and the middle includes areas between the lowest and highest water levels. Benthos and soil organisms, not plankton or periphyton, occur in the middle zone. In this sense organisms of sand filters and similar sewage treatment structures would belong to the hygropsammon. The " Schmutzdecke " of German workers with sand filters in water purification systems (74) also belongs here.

For micro-communities of algae, a series of terms applicable in some of the areas considered here has been proposed (67). Hydrophytes are more or less completely submerged and floating, and may include benthophytes growing on mud or other bottom of a body of water; epactiphytes grow along the shores of lakes or ponds; epiphytes grow on other plants, and epizoophytes grow on animals. Edaphophytes grow on the soil, and if they are completely or almost completely exposed they are saphophytes, or if they are subterranean they are crytophytes. Aerophytes grow on

structures extending from or above the soil and are not strictly soil forms; if they grow on plants they are epiphytes, if on leaves they are epiphyllophytes, and on bark epiphloeophytes; or if they are on animals they are epizoophytes, or on rocks lithophytes. Lithophytes are ". . . the algae growing on stones, boulders, cliff surfaces and other permanent rocky exposures. Habitat may vary from granite to sandstone, with slow or rapid erosion, from shade to intense sunshine, from trickling water to blistering heat and desiccation". Such terms, or modifications of them to fit the group of organisms under consideration, can be extended to such groups as the bacteria, fungi, and protozoans, as well as to larger animals and to such higher plants as mosses, liverworts, ferns and even flowering plants.

In the description of organisms occurring in sewage treatment plants and in polluted water, the term "microbiota" (76) has been chosen for " all organisms, plants and animals, from bacteria through certain aquatic insect larvae". This includes both benthos and plankton, the plankton occurring in the flowing sewage, the polluted stream, the aeration chamber and the digester; the benthos, including the biological film on the trickling filter bed or similar treatment unit, the walls of various types of tanks holding material in process of treatment, as well as the bottom organisms in a polluted stream. The microbiota of sewage treatment plants, especially of trickling filter beds, are further subdivided into " binding " organisms which form the filamentous-gelatinous matrix of the biological film, the " free-living " organisms which feed on bacteria and the dissolved materials in sewage, and the " scouring " or " grazing " organisms which feed directly on the film and the sewage solids. This classification of the benthic organisms can also be applied to polluted streams, especially in the vicinity of the effluent causing the pollution.

Since there is a tendency to limit terminology for clarity, the terminology described in the preceding paragraph can be amended as more and more becomes known of a habitat or of the groups of organisms inhabiting an area. A trickling filter is essentially a waterfall with the water falling over large quantities of stones or other similar material. The water contains planktonic and benthic organisms, derived from sewage and from surface run-off. Planktonic organisms may be carried away from the treatment plant in the effluent, or they may remain as facultative members of the ben-

thos. Since the filter substrate is made up completely of stone, the organisms of the habitat belong in the biocoenose of the Aufwuchs. If we include both epiphyton and periphyton in the Aufwuchs, then the organisms of the trickling filter slime are periphyton. These periphytic organisms can be differentiated as binding, free-living, and scouring or grazing organisms of the periphyton.

Summarizing, the binding, free-living and grazing or scouring organisms on the trickling filter stone form three interdependent communities within the periphyton of the Aufwuchs biocoenose of the benthos, and they include both true benthic and facultative benthic-planktonic organisms which may be sessile or sessile-attached to the substratum. Those that are sessile-attached are true lithophytes, lithozoons or lithobionts, depending on the type of organism under consideration.

TECHNIQUES

It is difficult to say when the organisms discussed above were first studied as a group. Organisms of the benthos, plankton, shore sands, soil and air have been studied for a long time. The first mention of a specific technique for the study of aquatic organisms by means of introducing foreign solid substances, onto which these organisms could adhere, was found in a Swedish publication (46) dated 1915. Prior to that time organisms which had become attached to various submerged materials had been studied, plating techniques for the study of water-borne and air-borne organisms were known, and various devices for trapping or netting organisms in water, soil and air had been devised.

In a footnote to a brief description of technique published in England, it was noted (30), concerning the slide-exposure technique: " I do not think it was an original operation, for I seem to remember having got the idea from something I had read ". Many of the techniques to be described below are simple or complex modifications of previously published techniques; others are reported as new techniques, for the writers appear to have been unfamiliar with previously published methods and thus may have genuinely invented a device similar to, if not identical with, other devices invented for the same or similar purposes. Various workers tried to adapt their techniques to a special group of organisms; others were more interested in finding or studying anything which could be obtained. The following discussion will be divided

according to the various general or specific groups of organisms in which the writers were interested. As a summary, it is probably incomplete, but it is thought to include most of the references in which techniques are described and results based on the techniques are given.

GENERAL STUDIES OF THE AUFWUCHS

One of the earlier methods of studying the organisms which become attached to submerged surfaces was direct examination of the adherent populations on very thin leaves, such as those of *Elodea*. By observing the leaf through ruled glass slides, quantitative estimates could be obtained (75) of populations, and the organisms involved could be identified. In addition to nets, traps were used to sample plankton in St. Moritz Lake (5). In a discussion (65) of techniques used for the study of organisms in various types of water, the *Elodea*-leaf technique was described. The exposing of "Gaslichtpapier" in aquatic habitats for obtaining various microorganisms for photomicrography was described (47, 48) in Sweden.

In describing the biocoenoses of Lunz Lake (6), those plant and animal communities which can be studied by direct observation of the stems and leaves of *Elodea* and *Chara* were listed.

The methods available for the study of microphytes in the littoral and profundal zones of lakes were described in 1926 (66). Diatom populations were divided into groups, those of the Aufwuchs occurring only on living stems, and those of other bottom habitats. The glass slide technique for their study was described with the indication that it was first used in 1915 (46).

To understand the technique and later modifications of it, the glass slide mounts first used by Hentschel (28) in Hamburg should be described. Glass slides 30 cm. × 15 cm. × ¾ mm. were divided with scratched lines on the upper surface into either 2 × 5 cm. oblongs, or 7.5 cm. squares. These slides were mounted in "pontoons" so that they hung vertically in water one and two meters below the surface. These were exposed for three, seven, and 14 days during a year's time. Reports were based on numbers of individuals per 100 square centimeters. Eight types of growth forms were recognized, including crust-forming protista, branch-forming protista, growth of short-stalked protozoa, cushion-forming protista, cushion-forming metazoa, and mussel growth.

A technique suggested from Nigeria (41) calls for placing two cover glasses together, tying them with thread, and exposing them in a forest pool in such a way that their base touches the bottom and they are in an inclined position. After suitable exposure the glasses are removed, and the protozoans and algae which have developed on them may be used for class demonstration purposes. Commenting on this (30), it was indicated that in England a similar technique had been in use for studying primordia and tests of protozoa in life history studies. It was suggested that in a natural situation several difficulties could be expected, as the washing effect of wind and rain and the unbalancing effect of marauding fish and other organisms feeding in water.

An instrument for securing marine invertebrates was described (4) in 1930. This was subsequently used in various modifications by several workers. The first use of the apparatus was credited to C. L. Parmenter who preceded its describer, Bissonnette, on the staff at Woods Hole. It is possible that the original instrument had been used as early as 1919. Two pieces of wood $34.5 \times 2.7 \times 1.5$ cm. and two pieces $7.0 \times 2.7 \times 1.5$ cm. were fastened together to form a rectangular frame $34.5 \times 10.0 \times 2.7$ cm. outside and $31.5 \times 7.0 \times 2.5$ cm. inside. Twenty saw cuts were made in the long pieces at opposite points to hold 20 3- × 1-inch slides. The two open faces of the frame were covered with 0.8-cm.-mesh zinc wire (hardward cloth), and one of these was hinged for ready access by the operator to the slides. After slides were mounted in the frames, these were hung in vertical pairs three feet apart on tarred fish-net cord, and a brick or stone sinker was attached five feet from the lower rack of a pair by a cord wound around the rack. The lower rack should not touch bottom. The racks were largely used for the collection of bryozoans for systematic, class and life history or experimental studies.

The Bissonnette rack, or a slight modification of it, was used in Michigan (44) for the study of the quantitative seasonal distribution of *Pelmatohydra* in a fresh-water lake. This carrier was described in a recent text on limnology (73), with comments on its limitations and advantages. Among the limitations mentioned are that it merely supplements other work, and that it is most useful in upper waters, since it can easily become contaminated on removal through various levels of water, and finally that only those organ-

isms which adhere or can become attached will be obtained in this way. Among the advantages of the technique are that the specimens are undamaged, they are kept alive easier, they can be kept alive in the refrigerator or in storage above freezing, that the method gives information about organisms with attached stages, it may aid in the collection of rarer species, it can be used in places where nets are used with difficulty, it gives information about succession, and that organisms can be killed and fixed in situ for future study.

It was found in Spain (43) that stones from the bottom of a stream could be processed using fixing and staining techniques, a collodium film applied and just before final drying peeled off in fragments. The fragments could be mounted in Canada balsam or other viscous media and studied quantitatively. Epilithic communities studied in this way were made up of bacteria, fungi, diatoms and green and blue-green algae. The first peel was shown to remove all organisms attached to or growing on the stone.

In a discussion of experimental populations of microorganisms it has been indicated (23) that the slide technique can be used to regulate the study of communities in their normal habitats. Since certain organisms develop with great regularity on exposed glass plates, some of them can be removed at will to determine the effect of such removal on the other organisms.

The periphyton of Douglas Lake, Michigan, was studied (83) by scraping measured portions of *Scirpus* and other stems submerged in the water. Materials on submerged stones as well as those on clean shore stones placed in the water were also studied.

In the study of the productivity of Sodom Lake, Michigan (52, 53), "attachment materials" were emphasized. In the earlier of these papers it was indicated that in a Bissonnette-type sampler four slides should be adequate for this type of study, since three were too few and six were more than enough. The period for submergence of the slides was about 25 days. The weight of organisms on a suitable, uniform, submerged surface is a somewhat more direct measure of the productive capacity of a body of water than other techniques. The method is sensitive to changing conditions within the lake. In the later paper, plastic and glass slides were used. Plastic slides were attached to a six-inch cube wood block, glass slides 2.5×7.5 cm. were placed horizontally, and glass slides 5.7×40.6 cm. were set vertically in the water. From a

comparative study made in late summer and early fall, it was found that in accumulation of materials the ratio of vertically to horizontally placed slides was 1:6.6, so the horizontal position was used in later work. Using another type of frame (44), three series of slides were exposed at each location in order to obtain data on loss by ignition, collections for a quantitative study of material suspended in glycerin, and material for a chemical study. Only "attachment materials" "seeded-on" naturally were studied. Mean weights of six slides were compared as to materials from the upper surface of the slide, the lower surface, and the combined surfaces. A formula was developed for "seeding-on" based on "compound interest at a constant rate". It was suggested that the equipment could be modified toward simplification.

PLANKTON: THE CONTINUOUS PLANKTON RECORDER

While plankton is not the subject of this review, it is possible that some organisms of the plankton may become attached to submerged surfaces. These organisms could be obtained by various types of plankton-trapping mechanisms such as nets, and by the various techniques described in the plankton literature. The continuous plankton recorder (22, 24) is such an instrument. This is a miniature submarine-like device in which a spool of gauze is made to unwind onto an empty spool seated in a tank of preservative, by means of a series of gears powered by a propeller in the rear of the device. As the machine is towed by a ship, water is drawn through an opening in the front, and the organisms it contains are impinged on the moving strip of gauze which can be marked according to the day and hour of exposure. The movement of the machine through the water spins the propeller which drives the gears. The exact location of the collecting device when any point on the gauze was exposed can be determined by reference to the ship's log. In this way records (1) have been obtained, and lists of different types of plankton and their relation to other marine life have been determined (12, 25, 42, 57).

DIATOMS

As a group of organisms distinct from other members of the plankton and periphyton, diatoms have received little attention as far as special techniques for obtaining them directly from the environment are concerned. A slide technique was adapted to the

study of these organisms on the California Coast (77). Wooden blocks at the surface and in deeper water, scraped surfaces, cleared rock surfaces both in pools and along the shore, and glass plates were exposed to colonization or recolonization. These techniques correspond to suggestions made as early as 1905 (14) concerning the study of communities, colonization and succession in cleared surfaces. Easiest to handle were glass plates enclosed in frames made to accommodate 30 3- × 4-inch glass plates, exposed at the surface of the ocean and ten feet below the surface (30 and 40 feet below the floor of the pier of the Scripps Institute) 900 feet from the shore. Daily observations and records were made of diatoms with holdfasts, of protozoans and of algae. The frames used appear to have resembled modified slide boxes or modified Bissonnette samplers, open at both sides, with slides in pairs. They were suspended so that all slides were horizontal. To illustrate uses for this technique, two studies may be mentioned. In one series of slides, *Cocconeis scutellum* was dominant, and the asymmetrical development and variation in this species was readily studied (78). In another series, *Navicula rhombica* formed colonies, and at daily intervals the development of these colonies was studied (79).

More recently (56), in the Philadelphia area, a somewhat different slide-carrying device was developed to sample the diatom populations of streams. The Catherwood diatometer consists of a plastic base mounted on a lead bar shaped like a boat. On the plastic base are mounted two floats designed so that the depth to which the diatometer is sunk can be varied. Between the floats, behind a plastic, V-shaped vane, is mounted the plastic slide holder slotted to hold six slides vertically, edgewise to the current. The vane prevents excess washing of the slides. It was stated that one week was sufficient time to expose the slides and that the total population of an unpolluted stream could be estimated as adequately with this method as with the usual methods of collecting diatoms. Calculations upon which these estimates are based have to be corrected when dealing with polluted streams.

SOIL MICROORGANISMS

In all phases of bacteriology and mycology, the use of plating methods to isolate organisms from habitats such as water supplies, soil samples and other test samples is a well-known basic tech-

nique. The individual worker may modify this technique by using a different size sample, different dilutions, different medium or other factor. However, as indicated in other types of samplings, this does not give a complete picture of all the organisms present. A number of techniques have been proposed to remedy this situation, among them exposure of various types of cleared surfaces, such as glass slides, to natural and disturbed samples. The following techniques may be mentioned as basic, although the list is admittedly incomplete.

The first application of direct microscopy to the examination of soils (17–20) was based on a technique borrowed from microtechnique. This involved drying a soil infusion on a slide, staining and observing the results. It was indicated that five to twenty times as many bacteria were reportable this way as with soil plating methods. This technique was modified in France by centrifuging (80–82) a soil sample, of which one fraction was then taken for mounting as a soil infusion. In Germany (20, 37, 38) this technique was adapted to a direct count of soil organisms, but with unsatisfactory results. An Italian modification for direct examination of soil organisms (60) utilized a glass slide placed in a special holder directly in the soil in a special cut area. The slide was removed after a period of exposure and stained. To the present time this is the most direct method suggested for study of soil organisms.

The Cholodny technique is simply a modification (13) of the Rossi technique (60), in which a trench is cut in the soil and a glass slide is placed against an undisturbed surface of the soil, covered and left for one to two weeks. At the end of this time the slide is removed, fixed, stained and observed. In removing the slide from the soil, care should be taken to prevent disturbance of the organisms on it. Practically all forms observed in Europe (13) with this technique were later observed (20) in New York State. Cholodny termed the slides exposed in this way " Bodenplatten " or " Aufwuchsplatten ". The technique is not a quantitative one in the usually accepted sense, but when used properly it can give qualitative or physiognomic data.

In a modification of this technique suggested in New York (20), the soil to be studied is brought from the field to the laboratory and placed in a covered jelly glass or similar container with

two glass slides which are incubated in the soil for five to seven days. The length of time can be varied, and a new slide can be added when one is removed. In this way one can study colonies in pure culture, determine deficiencies in the populations of various soils, and determine the effect of fertilization or moisture on soil populations. The technique has certain disadvantages in that no two slides can be placed in the same population, and identification of species appearing on slides is difficult if not usually impossible. The exact technique for using slides in this way must still be perfected. Workers in Czechoslovakia (39) and the United States (71) have summarized studies in which this technique was used. It appears that up to 1952 little use has been made of it beyond the adaptation of a microscope (39) for direct examination of soil. For direct microscopic study of soil, the examination of individual grains of soil for their micro-populations is now considered the best technique. Types of soil examinations beyond the use of direct exposure of glass slides are outside the scope of this paper.

FRESH-WATER, SALT-WATER AND MARINE BACTERIA

As long ago as 1925 (49) it was found that because of the tenacious attachment ability of iron bacteria, excellent preparations of them may be made by submergence of glass slides in iron-bearing waters. This is one of the earlier attempts to study bacteria in natural habitats by glass slide exposure.

Henrici (26) was the first to publish a technique using glass slides exposed in bodies of water for collecting bacteria for population studies. However, later (27) he gave priority credit to a Russian worker. It was felt that usual methods of isolation using selective media and the techniques of sanitation bacteriologists were inadequate, and a search was made for a more adequate ecological study tool. Henrici strung a series of glass slides at intervals along a line attached to a float anchored in a body of water. The line, selected to be of material not attractive to bacteria or other microorganisms, was two-millimeter rubber-coated copper radio wire. To prepare the slides, one-inch surgical adhesive tape cut in two and one half inch lengths was used. One end of a clean glass slide was heated and applied to the adhesive side of the tape so that one-quarter inch of the slide overlapped the tape. The tape

was then folded over on itself to one-quarter inch on the slide and a hole was punched in the one-inch square tab produced. The tab was numbered and dipped in melted paraffin before being used to fasten the slide to the anchor line with a short length of similar wire. No observations were made of differences in depth or in position (vertical or horizontal) of the slides. Duplicate slides could be used for the study of selected organisms. The slides were stained with bacteriological stains and examined. Density of bacteria increased with duration of exposure. The technique was thought to indicate the numbers of organisms brought to the slides and their rate of reproduction, so was regarded as a survey method. For quantitative studies, several refinements in the technique were suggested (*27*), based on comparisons of plating methods and slide exposures. It was specified that each observation should be made on two slides, 50×75 mm., suspended back to back; the duration of exposure should be adjusted to the habitat; two observations of differing duration should be made for each habitat; counting 100 or more organisms per field would give approximately 13,000 organisms per square millimeter of exposed surface; and that 50 fields per slide should be counted in the central part of the slide.

The early idea for the use of slides in studies of water bacteria in Russia (34) was obtained from Cholodny's Bodenplatten described above, as well as from the latter's "Aufwuchsplattenmethode" for the study of water organisms. For slides exposed in water, wood frames were avoided because of the possibility of contamination of slides by wood-decay organisms. Tin-plated steel carriers suspended from buoys were used for water exposures. Only the outer half of the slide was used for study, to avoid possible deleterious effects of metallic ions. For bottom samplers, wood frames were used, consisting of flat pieces of wood 20×25 cm. with triangular curbs two cm. high on the long sides of each piece. In six slots in each of the four curbs, slides were inserted. Of these 24 slides, 12 were exposed in the ooze and muck of the bottom of the body of water sampled, and the remaining 12 were exposed to the water itself. Between the curbs on the upper side of the frame was fastened a sinker which was suspended from a buoy by a rope. Glubokoje Lake was sampled at depths of 1.5, 3.5, 5.5, 8, 10, 14.5 and 15 meters. Early in September, three-day

exposures were used; in mid-September and late September, one- and three-day exposures were used. Toward the end of September exposures of seven, 14, 21 and 28 days were tried. Slides removed for study were dried at room temperature, fixed in alcohol, stained 30 to 40 minutes in 5% erythrosin and carbolated water, rinsed and examined. Organisms, bacteria, fungi, algae and protozoans were reported.

Several bodies of water in Russia were studied (35) for their bacterial content by workers using the techniques described by Henrici. The warmer waters required only 12 to 24 hours of exposure for an adequate deposition of bacteria on slides, while in cold water longer periods were required. Results could be correlated with the degree of pollution when chemical analyses of water were known. Various levels in the bodies of water studied were used, but no significant differences in collections were found. At least two days were required to obtain results with coliform bacteria, which became attached to slides with difficulty, and therefore plating methods were considered preferable for studying those organisms.

Studies (86) of the attachment of bacteria and diatoms to slides were carried out in California in relation to fouling. It was noted that bacteria, diatoms and actinomycetes became attached to glass plates several days before fouling organisms did. Not all marine bacteria became attached to slides, and laboratory experiments indicated that 24 of 73 cultures used formed films on slides before the water in which the culture was growing became clouded, while three of these became attached simultaneously with clouding of the water. On the other hand, use of exposure plates developed the presence of certain bacteria which could not be obtained by dish plating methods. In culture it took two to four hours for the bacteria to become attached to slides in appreciable numbers. All stages in their development were observed on the slides. Holdfast material was neither water-soluble nor alcohol-soluble; some of it could be dissolved with xylol. The technique was good only for the study of "attachment" bacteria.

Using criticisms previously found valid by other workers, it was observed (70) in a semi-popular review of the subject that the direct-slide method for the study of marine bacteria was incomplete as a survey tool because distinctions between live and dead

cells could not be made, and because some bacteria do not become attached to glass surfaces.

Improvements in the technique of studying marine bacteria by exposure of glass plates were described (87) from California studies. The carrier used was $12 \times 4 \times 0.25$ inch, made of lead covered with wood, coated with paraffin. The wooden bottom was grooved to hold slides vertically. Twelve to 16 3- × 1-inch glass slides were placed in the carrier aseptically, held in place with cord, and suspended with a cotton rope in six to 12 feet of water. The slides were exposed from a few hours to a few days. Study of exposed slides was practical after only a few hours, as the longer exposures brought up so many organisms that the slides became increasingly difficult to study. Observation of the slides was by direct bacteriological techniques. Such a study obtains information on the primary film-formers; these organisms favor the later colonization by other, larger fouling organisms.

To the extent that the writers were interested in slide exposure techniques and the results obtained from them, reviews have appeared (72, 85) in texts dealing with limnology and the study of marine life.

The " attachment count " was compared (32) with plate counts in samples of sea water in the laboratory. Samples with small numbers of bacteria were used. Experiments were designed to show the influence of temperature on attachment, the influence of nutrient added to the slides, the attachment of pure cultures, the influence of filtration and the effects of light. It was suggested that statistical constants between the attachment and plate count methods could be helpful, but that the plate count method would be easier and more readily reproducible in most areas.

The native bacteria of the Great Salt Lake were studied (64), using glass slides. Four points, distant from the introduction of surface run-off and pollution to the lake, were used for this study. Wooden carriers, holding up to 12 slides, were supported at the top with a cord. The whole apparatus, with its anchor cords, was boiled in paraffin before loading, the slides were set in place aseptically, and the equipment was submerged for 12 hours to five weeks. Best results were obtained in 24 hours, since slides exposed for longer periods were subject to extensive corrosion and to heavy deposition of salt. Such corrosion and deposits made ob-

servation of microorganisms difficult but not completely impossible. The experiments showed that bacteria from sewage, soil or the ocean were not viable in Great Salt Lake water. Tap water damaged the native cells which were found. The actively growing and micro-colony-forming bacteria observed on the slides were part of the normal population of the Lake.

The effect of solid surfaces on bacterial activity was studied (84) in California. Glass, plastics, porcelain, sand, kieselguhr and other inert particulate materials are surface-active: particles smaller than bacteria may be harmful; particles larger than bacteria may be beneficial. Glass surfaces adsorbed minute but demonstrable quantities of organic nutrients, and, since sea water is a very dilute nutrient medium, its concentration by adsorption on the glass slides enhanced the development of bacteria. Those marine bacteria which can be sessile secrete a mucilaginous holdfast which may also adsorb nutrient materials from the sea water. These beneficial effects appear only when the nutrients were less than ten p.p.m., and the benefits are more pronounced when the nutrients are colloidal or poorly soluble instead of readily soluble. Solid surfaces retard the diffusion of exoenzymes and hydrolysates promoting the assimilation of nutrients.

FOULING ORGANISMS

A very important problem to man arises from the fouling of ship bottoms, marine shellfish, piers and other structures exposed for prolonged periods in ocean waters. Attempts have been made to find suitable paints that would inhibit the growth of organisms that become attached to the bottom of vessels. Various studies have been made of the organisms that cause fouling, and of their life cycles, in order to devise some means of successfully combatting them. In recent years a number of studies have been made with different types of surfaces exposed for varying periods to the activity of these organisms.

As early as 1924, aluminum, zinc, iron, tin, lead and copper plates, 15 cm. square, as well as 15 combinations of these metals hammered together to form 15-cm.-square plates, were exposed (55) to test the ability of marine animals to use such surfaces. The plates were exposed at Eel Pond, Woods Hole, Mass., early in July and removed in late August or early September. The

plates were suspended in a vertical position in a floating wooden frame with the upper part of the frame 20 cm. below the water surface. Animals did not attach themselves to copper, combinations of copper and lead, or any combination in which zinc was used. Few came on zinc alone. On the other metals and combinations of metals, at least one-fifth of the surface was covered, but mostly three-fifths to all of the surface was covered. Very little algal or microscopic growth was noted.

Using wood and cement cubes to determine the season of attachment and rate of growth of sedentary organisms, observations were made (17) throughout the year at La Jolla, California, at four- to 20-week intervals. On the wood blocks, 66 sq. in. of surface were exposed; on the cement blocks, 28 sq. in. Only macroorganisms were studied. In all months except in winter all organisms became attached to some degree. Most fouling occurred during the reproductive season, June to September. Tabulations of the numbers of barnacles fouling wood and cement surfaces showed that more became attached to cement surfaces than to wood. Rate of growth of barnacles, scallops and oysters as well as season of reproductive activity were determined in this study.

The attachment of larvae of the Olympia oyster to plane surfaces has been investigated at Friday Harbor, Washington (31). Frames of one-half-inch-mesh hardware cloth were used, each holding three 8- × 10-inch plates of clear window glass. The pieces of glass were held one meter apart, but the frames were so wired that the plates could be placed at any desired angle. Cement-covered cardboard, arranged so that both horizontal and vertical faces were available to the animals, were also used. It was found that, because of the upward orientation of the basal foot of the larva, the under side of horizontal glass surfaces was more important than the vertical even when the glass was made opaque with black paint.

Blocks, $11 \times 5\frac{1}{2} \times \frac{3}{4}$ inch, cut from Douglas-fir boards were used (33) to test the seasonal activity of certain fouling organisms at Friday Harbor. The blocks were mounted rigidly on a cable suspended from a pier, two meters from pilings and anchored at the bottom. The blocks were placed at the known levels of greatest activity of organisms fouling the pier pilings. With reference to the zero tide datum, they were placed at depths of 6.3, 3.3. 0.3

and –2.7 feet. The blocks were overlapped in timing and removed at three-month intervals, those placed Oct. 1 being removed Jan. 1, those placed Nov. 1 being removed Feb. 1, etc. During the year, the season of settlement for barnacles, shipworms, tubiculous annelids and bryozoa was obtained.

Periodically cleaned glass slides were used to supplement earlier exposure slides using glass plates in California (16). Such studies were designed to identify seasonal activities and other periodicities of the sedentary populations; to determine the life cycles and life histories of the different components of the population; to determine the more common or important sequence of occurrence of the population; identify ranges of tolerance of adverse environmental conditions; learn something of the interrelations between sedentary, plankton and nekton organisms; and to increase knowledge of the general relation of some of the populations to discernible conditions of the environment peculiar to the marginal zones of the ocean. A frame of galvanized angle iron with channeled cross bars was devised to hold six 8- × 9-inch glass plates. The device was attached to a boom and anchored so that it could be held rigidly. It was removed at four-week intervals during a three-year period. One side of one plate was scraped with a razor blade at each removal, and a clean glass was added so that growth could be compared on clean glass, scraped glass and unscraped glass. At the end of four weeks growth was more luxuriant on the scraped glass than on the new glass, although it had not caught up with the unscraped glass. The better growth on the scraped glass was attributed to the retention of micro-organisms and minute particles from the material scraped off. The amount of growth was roughly correlated with mean surface temperature. The outer face of the plates had more growth than the inner, but speciation was not compared.

Ten groups of organisms which caused fouling on oyster shells in suspended oyster beds in Japan were studied (45). Calcareous plates 15 cm. square and 0.5 cm. thick were used. Nine plates were suspended in a unit, three in each of three sets. The sets were spaced 100 cm. apart on a cable, the plates in each set being 15 cm. apart. The units were placed horizontally in upper, middle and lower water. Plates were removed at the end of two months and replaced with clean plates. This period of time was satisfactory for the locality. Some of the organisms serve as fish food.

In a study (21) of fouling organisms in waters in Hawaii, five glass plates, 3×8 inches, mounted vertically in a wood frame 19 $\times 9\frac{1}{2}$ inches supported by ropes were used. For a three-year period studies were made of the development of such groups of fouling organisms as barnacles, bryozoa, serpuloid worms and mollusks. Particular interest was shown in the effects of this growth on various metals—iron, tin, galvanized iron, lead, monel, German silver, brass, copper and zinc. Various non-toxic paints, non-toxic paints with added poisons, and commercial antifouling compounds were applied in an attempt to find a way to prevent fouling. The best technique was rarely effective for more than six months.

Sand-blasted glass panels 3×4 inches, painted on both sides, mounted in a wooden frame and submerged were used (36) in an effort to determine an effective agent for preventing fouling. A copper-base paint releasing copper to the water at a definite toxicity rate was effective against macroorganisms causing fouling. Since microorganisms were not considered to be a problem in fouling, the lack of effect of this paint on them was not considered important.

A technique used for collecting shipworms, and adaptable to any type of ecological study, has been described (69). A number of boards, $12 \times 6 \times 1$ inches, are set two inches apart by using brass bolts, nuts, washers and screws. This rig is immersed in the body of water to be tested. At the end of one month one board is removed and replaced with a new board. At the end of two months one of the original boards is removed together with the one added at the end of the first month, and a new board is added. This process can be repeated as many times as there were boards in the initial set.

SEWAGE ORGANISMS

The use of slides in the study of sewage organisms has largely been confined to England. However, it was suggested by a worker in Hamburg, Germany (29), that glass slides or plates could be suspended in water by using weights below and buoys above to anchor them. An alternate method was to take a standard cement brick with two parallel tubes through it, place glass slides or other appropriate objects in these tubes, and study the organisms which appear.

On the beds of streams under study in England, where pollution consisted largely of industrial wastes, glass slides in frames were used as early as 1932 (8) for study of algae. This was an elaboration of an earlier technique (10) described more fully in another paper (7). Five glass slides $1 \times 3\frac{1}{2}$ inches were mounted in photographic frames laid on the bed of a stream and held in place with brass chains attached to stakes. Four slides were used in quantitative studies of the weight of material accumulated; the fifth was used for identification of the organisms obtained. The slides were exposed for only 14 days, but longer periods would have been preferable. The method was preferred to one in which stones are scraped, since in the latter method a number of species and stages are missed.

In studying the populations and changes in populations on percolating filters operated alternately in series, glass slides were used (68). Sets of six 3- × 1-inch glass slides exposed in photographic film holders were weighed periodically. These weights were comparable with weights of slime on stone. The growth on the slides was sensitive to three to four day and seven to 14 day alternations in filter operation periods. Changes at one-foot depths in the filters followed the same pattern when slides and pieces of coke were used in comparison with slides and stone of the filtering medium on the surface.

In studying (58) the biology of a double filtration plant in England, two-inch glass squares with roughened surfaces were paired with wood blocks four to six inches below the surface of the filter. The blocks had 56 square inches of exposed surface. For the type of information desired, it was thought that the wood blocks were better than the glass plates.

In a study (9) of the biological detection of sewage, data from exposed slides were used in tabular form. Data averaged from six slides were reported as average numbers of organisms per square millimeter of surface after 21 days exposure.

CONCLUSION

This summary indicates that glass slides and plates and other materials, such as pieces of wood and cement blocks, have been used to advantage as bare areas in the study of the initiation and development of communities of organisms which grow attached to

surfaces of many types in aquatic environments, as well as for the study of organisms occurring in the soil. The method of exposing the slides and the type of carrier developed for them has varied with the type of habitat and organisms studied, or with the anticipated effect of various carrier materials on the population. Criticisms of the techniques described as techniques designed to obtain all organisms of a habitat are about as valid as criticisms of one medium designed to obtain all organisms by cultural methods, since no one medium or technique can do this. It is apparent that the techniques are highly adaptable, and that from them can be gained information concerning types of organisms capable of adhering to a surface, their quality, density, rate of development, succession of communities and the component members of communities in aquatic environments.

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BIBLIOGRAPHY

1. Anon. 1937. Summary list of records. Hull Bull. Marine Ecol. **1**(2): 59–71.
2. Behning, A. L. 1924. Zur Erforschung der am Flussboden der Wolga lebenden Organismen. Monogr. Biol. Wolga. Statt. **1**: 1–398.
3. ————. 1928. Das Leben der Wolga. Vol. 5. Die Binnengewässer.
4. Bissonnette, T. H. 1930. A method of securing marine invertebrates. Science **71**(1844): 464–465.
5. Borner, L. Die Bodenfauna des St. Mortizer:Sees. Eine monographische Studie. Arch. Hydrobiol. **13**: 1–91, 209–281. Tab. 1, 2.
6. Brehn, V., and F. Ruttner. 1926. Die Biocoenosen der Lunzer Gewässer. Int. Rev. Ges. Hydrobiol. & Hydrogr. **16**(5–6): 281–391.
7. Butcher, R. W. 1932. Studies on the ecology of rivers. II. The microflora of rivers with special reference to the algae on the river bed. Ann. Bot. **46**: 813–861.
8. ————. 1932. A method of investigating the algae on the bed of a river. Trans. North. Nat. Union. i. 1932.
9. ————. 1946. The biological detection of pollution. Inst. Sew. Purif. Midland Meeting. Pp. 3–8.
10. ————, F. T. K. Pentelow, and J. M. Woodley. 1927. Diurnal variation of the gaseous constituents of river waters. Biochem. Jour. **21**: 945, 1423.

11. CHAPMAN, V. J. 1941. An introduction to the study of algae. 387 pp.
12. CHENG, C. 1941. Ecological relations between the herring and the plankton off the north-east coast of England. Hull Bull. Marine Ecol. **1**(5) : 239–253.
13. CHOLODNY, N. 1930. Über eine neue Methode zur Untersuchung der Bodenmikroflora. Arch. Mikrobiol. **1**: 620–652.
14. CLEMENTS, F. E. 1905. Research methods in ecology.
15. COE, W. R. 1932. Season of attachment and rate of growth of sedentary marine organisms at the pier of the Scripps Institution of Oceanography. Bull. Scripps Inst. Ocean., Univ. Calif., Tech. Ser. **3**(3) : 37–86.
16. ———— and W. E. ALLEN. 1937. Growth of sedentary marine organisms on blocks and plates for nine successive years at the pier of the Scripps Institution of Oceanography. Bull. Scripps Inst. Ocean., Univ. Calif., Tech. Ser. **4**(4) : 101–136.
17. CONN, H. J. 1917. The direct microscopic examination of bacteria in soil. Abstr. Bact. **1**: 40.
18. ————. 1918. The microscopic study of bacteria and fungi in soil. N. Y. Agr. Exp. Sta., Tech. Bull. **64**: 1 et seq.
19. ————. 1932. The Cholodny technic for the microscopic study of the soil microflora. Zent. Bakt., Parasit. & Infekts. II. **87**: 233–239.
20. ————. 1932. A microscopic study of certain changes in the microflora of soil. N. Y. Agr. Exp. Sta., Tech. Bull. 204.
21. EDMONDSON, C. H., and W. M. INGRAM. 1939. Fouling organisms in Hawaii. Occas. Pap. Bernice P. Bishop Mus. **14**(14) : 251–300.
22. ELTON, C. 1940. Review. Jour. Animal Ecol. **9**: 338–339.
23. GAUSE, G. F. 1937. Experimental populations of microorganisms. Ecology **18**: 173–179.
24. HARDY, A. C. 1939. Ecological investigations with the continuous plankton recorder. Hull Bull. Marine Ecol. **1**(1) : 1–57.
25. HENDERSON, G. T. D., and N. B. MARSHALL. 1944. Ecological investigation with the continuous plankton recorder: zooplankton (other than copepods and young fish) in the southern North Sea, 1932–37. Hull Bull. Marine Ecol. **1**(6) : 255–275.
26. HENRICI, A. T. 1933. Studies of freshwater bacteria. I. A direct microscopic technique. Jour. Bact. **25**: 277–286.
27. ————. 1936. Studies of freshwater bacteria. III. Quantitative aspects of the direct microscopic method. Jour. Bact. **32**: 265–280.
28. HENTSCHEL, E. 1916. Biologische Untersuchungen über den tierschen und pflanzenlichen Bewuchs in Hamburger Hafen. Mitt. Zool. Mus. Hamburg **33**: 1–172.
29. ————. 1923. Abwasserbiologie. Aberhalden, Handb. Biol. Arbeitsmethoden. **9: 2: 1: 1**: 233–280.
30. HERON-ALLEN, E. 1930. [Note]. Nature **125**: 91.
31. HOPKINS, A. E. 1935. Attachment of largae of the Olympia oyster, *Ostrea lurida*, to plane surfaces. Ecology **16**: 82–87.
32. HOTCHKISS, M., and S. A. WAKSMAN. 1936. Correlative studies of microscopic and plate methods for evaluating the bacterial population of the sea. Jour. Bact. **32**: 423–432.
33. JOHNSON, M. W., and R. C. MILLER. 1935. The seasonal settlement of shipworms, barnacles, and other wharf-pile organisms at Friday Harbor, Washington. Univ. Wash. Publ. Oceanogr. **2**(1) : 1–18.
34. KARSINKIN, G. 1934. Zum Studien des Bacterialen Periphytons. Arb. Limnol. Sta. Kossino **17**: 21–44. [Russian with German summary].
35. ———— and Z. I. KUSNETZOWA. 1934. Erforschung das Bacteriellen Periphytons in Gewässern mit verschiedengradigen Verunreinigung. Arb. Limnol. Sta. Kossino **18**: 91–103. [Russian with German summary].

36. KETCHUM, B. H., J. D. FERRY, A. C. REDFIELD, and A. E. BURNS, JR. 1945. Evaluation of antifouling paints by leaching rate determinations. Ind. & Eng. Chem. **37:** 456–460.
37. KOFFMAN, M. 1926. Ein Methode zur direkten Untersuchung der Mikrofauna und der Mikroflora des Bodens. Centr. Bakt. II. **75:** 28–45.
38. ————. 1931. Weiteres zur Methode der direkten Untersuchung der Mikrofauna und Mikroflora des Bodens. Centr. Bakt. II. **85:** 1–11.
39. KUBIENA, W. L. 1938. Micropedology. 243 pp.
40. LENZ, F. 1928. Biologie der Süsswasserseen. Biologische Studienbücher IX.
41. LLOYD, L. 1930. Preparation of protozoa and algae. Nature **125:** 91.
42. LUCAS, C. E. 1940. The phytoplankton of the southern North Sea, 1932–37. Hull Bull. Marine Ecol. **1**(3) : 73–169.
43. MARGALEF, R. 1948. A new limnological method for the investigation of thin-layered epilithic communities. Trans. Amer. Micr. Soc. **47:** 153–154.
44. MILLER, D. E. 1936. A limnological study of *Pelmatohydra* with special reference to their quantitative seasonal distribution. Trans. Amer. Micr. Soc. **55:** 123–193.
45. MIYAZAKI, I. 1938. On fouling organisms in the oyster farm. Bull. Japan. Soc. Sci. Fish. **6:** 223–232.
46. NAUMANN, E. 1915. Skrifter utg. av. S. Sveriges Fiskeriförening. 1915.
47. ————. 1923. Die Untersuchung bestimmter Gewässern. See und Teich (Plankton und Neuston). Aberhalden, Hand. Biol. Arbeitsm. **9: 2: 1: 1:** 139–228.
48. ————. 1925. Untersuchungen über einige sub- und elitorale Algenassoziationen unserer Seen. Arkiv Bot. **19**(16) : 1–30.
49. ————. 1925. Wasserwerkbiologie. Aberhalden, Handb. Biol. Arbeitsm. **9: 2: 1: 1:** 229–232.
50. ————. 1931. Limnologische Terminologie. Aberhalden, Hanb. Biol. Arbeitsm. **9:** 8 : 1–5 : 1–776.
51. NEEL, J. K. 1948. A limnological investigation of the psammon in Douglas Lake, Michigan, with special reference to shoal and shoreline dynamics. Trans. Amer. Micr. Soc. **67:** 1–53.
52. NEWCOMBE, C. L. 1949. Attachment materials in relation to water productivity. Trans. Amer. Micr. Soc. **58:** 355–361.
53. ————. 1950. A quantitative study of attachment materials in Sodon Lake, Michigan. Ecology **31:** 204–215.
54. ODUM, E. P. 1953. Fundamentals of ecology. 384 pp.
55. Parker, G. H. 1924. The growth of marine animals on submerged metals. Biol. Bull. **47:** 127–134.
56. PATRICK, R., M. H. HOHN, and J. H. WALLACE. 1954. A new method for determining the pattern of the diatom flora. Notulae Naturae **259:** 1–12.
57. RAE, K. M., and J. H. FRASER. 1941. The Copepoda of the southern North Sea. Hull Bull. Marine Ecol. **1**(4) : 171–238.
58. REYNOLDSON, T. B. 1942. Further studies on the biology of a double filtration plant at Huddersfield. Inst. Sew. Purif. Ann. Gen. Meeting. Separate. Pp. 1–22.
59. ROLL, H. 1939. Zur Terminologie des Periphytons. Archiv. Hydrobiol. **35:** 59–69.
60. ROSSI, G. M. 1928. Il terreno agrario nella teoria e nella realta. L'Italia Agr. No. 4.
61. RUTTNER, F. 1953. Fundamentals of limnology. 242 pp. [Translated by D. G. Frey and F. E. J. Fry].

62. SELIGO, A. 1905. Über den Urspring Fischnahrung. Mitt. Westgr. Fisch. -V., Danzig. Mitt. **17**: 52–56.
63. SMITH, G. M. 1951. Manual of phycology. 375 pp.
64. SMITH, W. W., and C. E. ZOBELL. 1937. Direct microscopic evidence of an autochthonous bacterial flora in Great Salt Lake. Ecology **18**: 453–458.
65. THIENEMANN, A. 1923. Die Untersuchung bestimmter Gewässer: See und Teich, Ufer. Aberhalden, Handb. Biol. Arbeitsm. **9**: 2: 1: 1: 97–102.
66. THOMASSON, H. 1926. Methoden zur Untersuchung der Mikrophyten der limnischen Litoral- und Profundalzone. Aberhalden, Handb. Biol. Arbeitsm. **9**: 2: 1: 4: 681–712.
67. TIFFANY, L. H., and G. M. SMITH. 1951. Ecology of fresh-water algae. Pp. 293–311.
68. TOMLINSON, T. G. 1941. "Biological investigations" in the treatment of settled sewage in percolating filters in series, with periodic change in the order of the filters. Jour. & Proc., Inst. Sew. Purif. **1941**: [reprint pagination 1–64].
69. TURNER, R. D. 1947. Collecting shipworms. Limnol. Soc. Am., Sp. Publ. **19**: 1–4.
70. WAKSMAN, S. A. 1934. The role of bacteria in the cycle of life in the sea. Sci. Mont. **38**: 35–49.
71. ————. 1952. Soil microbiology. 356 pp.
72. WELCH, P. S. 1935. Limnology. 471 pp.
73. ————. 1948. Limnological methods. 381 pp.
74. WHIPPLE, G. C., G. M. FAIR, and M. C. WHIPPLE. 1948. The microscopy of drinking water.
75. WILLER, A. 1920. Über den Aufwuchs der Unterwasserflora. Schrift. Physik.-Ökonom. Ges. Königsberg. i. Pr. 61/62: 55–65.
76. WILSON, J. N. 1949. Microbiota of sewage treatment plants and polluted streams. *In*: Limnological aspects of water supply and waste disposal. [Reprint pagination 1–15].
77. WILSON, O. T. 1925. Some experimental observations of marine algal succession. Ecology **6**: 303–311.
78. ————. 1927. Asymmetrical variation in *Cocconeis scutellum*. Am. Jour. Bot. **14**: 267–273.
79. ————. 1929. The colonial development of *Navicula rhombica* Greg. Am. Jour. Bot. **16**: 825–831.
80. WINOGRADSKI, S. 1924A. Sur l'étude microscopique du sol. Comp. Rend. Acad. Sci. [Paris] **179**: 367.
81. ————. 1924B. La methode directe dans l'étude microbiologique do sol. Chim. et Ind. **2**(2).
82. ————. 1925. Études sur la microbiologie du sol. Ann. Inst. Past. **39**: 1–56, 299–354.
83. YOUNG, O. W. 1945. A limnological investigation of periphyton in Douglas Lake, Michigan. Trans. Am. Micr. Soc. **64**: 1–20.
84. ZOBELL, C. E. 1943. The effect of solid surfaces upon bacterial activity. Jour. Bact. **46**: 39–56.
85. ————. 1946. Marine microbiology. 240 pp.
86. ———— and E. C. ALLEN. 1933. Attachment of marine bacteria to submerged slides. Proc. Soc. Exp. Biol. & Med. **30**: 1409–1411.
87. ———— and ————. 1935. The significance of marine bacteria in the fouling of submerged surfaces. Jour. Bact. **29**: 239–251.

RECENT TRENDS IN PALYNOLOGY

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PREFACE

The term " palynology " was coined by Hyde and Williams [1] to cover all work with pollen grains and spores. The term is convenient and has been widely accepted, but it must not be taken to indicate that palynology should have a status as a science of its own. This it is not: pollen grains and spores are used in a wide variety of investigations in pure and applied botany, recent and fossil, which have practically nothing in common except the fact that pollen and spores are used as instruments of research. In that respect palynology resembles microscopy: the microscope is also used as an instrument in a great variety of unrelated investigations. There is a special science of the microscope, which is a branch of optics, and there is a special science of the pollen grain, viz., pollen morphology, which is a branch of plant morphology. The difference is that, whereas " microscopy " means the application of the microscope, " palynology " comprises both the science itself, i.e., pollen morphology, and its application.

Palynology is a rather heterogeneous subject, and the establishment of a separate section of palynology at the VIII International Botanical Congress (Paris, 1954) can hardly be described as an unqualified success. This failure does not reflect unfavourably on the French organizing committee which had done very good preparatory work, nor was it due to the loss of time spent in discussions of administrative problems. The inherent weakness was, I think, the heterogeneity of the subject. The much smaller palynological symposium arranged by Dr. Erdtman in connection with the VII International Botanical Congress (Stockholm, 1950) was very successful. Similar international symposia ought to be arranged informally in the future for discussing technical problems. Both in Europe and in the United States informal palynological meetings with a restricted membership have taken place more or

[1] Originally published in the privately circulated " Pollen and spore circular " nr. 8, 1943.

less regularly since the war. To those present, they have meant a great deal in keeping abreast of recent technical progress.

In contrast to many other papers in the Botanical Review, this one does not make any attempt at a complete survey of recent literature. Thanks to Dr. Erdtman, palynology is in the fortunate position of possessing an almost complete bibliography, even prior to the first days of pollen analysis and kept up-to-date since 1927.

In the following I shall therefore mention only those papers which I quote specifically. On the other hand, I shall include investigations which have so far been only incompletely published, or not at all, but which I have had the opportunity to learn about in connection with visits in the respective laboratories, including, during the summer of 1955, also some of the laboratories in USSR.

Expressing my thanks to all those colleagues who have received me in their laboratories, I want to thank especially the editor of The Botanical Review for the painstaking care with which he has revised the language of this contribution.

TEXT-BOOKS

Owing to the heterogeneity of the subject, there will probably never be a comprehensive text-book of palynology. In pollen morphology there are two outstanding works—Wodehouse's "Pollen grains" (1935), already a classic; and Erdtman's "Pollen morphology and plant taxonomy", of which the first volume appeared in 1952 and the second is, we hope, soon to follow. While the thorough discussions of the theoretical aspects constitute the most important part of the former work, the value of the latter lies in its great number of descriptions and illustrations of pollen from different families of plants. Descriptions and illustrations follow a very definite and very exhaustive plan, and are based upon the extensive collection of pollen preparations in the palynological laboratory at Bromma, near Stockholm (Sweden).

Erdtman's book is a world-embracing pollen flora. Regional pollen floras have been or are being prepared for a number of countries. Among the more outstanding should be mentioned Selling's from Hawaii, van Zinderen Bakker's from South Africa, and Cranwell's from New Zealand. I am also informed that Hyde is preparing a British pollen atlas. Otherwise, European pollen

has been neglected, the existing European floras being out-dated. We should also note monographic treatments of different taxonomic groups, e.g., Mrs. Knox' of Lycopodinae spores, Mme. van Campo's of gymnosperms.

With regard to the applications of palynology, few branches are adequately covered by text-books. Melittopalynology is comparatively favourably situated with a number of comprehensive works, of which Zander's should be especially mentioned, even though it has been instrumental in leading melittopalynological technique into what I consider an unfortunate direction. In Quaternary pollen analysis Fægri and Iversen (1950) published a text-book discussing principles, but giving no pollen pictures. Erdtman in 1943 published a text-book dealing more briefly with the problems of pollen analysis, and giving, at the same time, a shorter pollen atlas than his later, comprehensive work. This text-book has appeared in a second edition.

" Recent trends " in the title of this article is intended to cover partly the trends apparent in the modern text-books, partly in some later publications, and partly also in unpublished discussions. Palynology has many other aspects than those dealt with below. Work is in steady progress in many fields, and the exclusion of these from the present article does not mean that important results are not being achieved in them. As far as I am aware, there have in these fields not been any definitely new trends, no really new lines of investigation, in the last ten years.

POLLEN MORPHOLOGY

Wodehouse has pointed out that, since pollen grains are so small as to present features at or beyond the limit of resolution of the light microscope, each improvement of the microscope has been followed by a corresponding advance in our knowledge of their morphology. The last major improvement in microscope construction was the introduction, in 1884, of the apochromatic lens, followed in 1889 by Fischer's masterly work on pollen grains.

During the following 50 years progress was rather slow, and even to-day we cannot with the best visible-light microscope discern anything definitely new which was not seen by Fischer. The introduction of phase-contrast microscopy has not materially changed the situation. Pollen grains are, on the whole, not very

well suited for phase-contrast work, even though that technique brings out more clearly features that are otherwise difficult to detect, especially in unstained grains. The staining methods which have so far proved most satisfactory are also very simple and old-fashioned. Use of staining in pollen morphology is still a matter of some dispute. What can be seen in a stained preparation, can generally also be seen in an unstained one, but it is much easier to see it, and especially to detect it, in the former. Discussions concerning stained vs. unstained preparations, and similar questions of preparation technique, have so far been effective barriers against the establishment of an international agency for the exchange of pollen preparations.

"Pollen morphology" among palynologists refers to the morphology of the pollen exine. Most investigators either work with pollen that has been fossilised by natural means, and consequently has lost intine and protoplasmic contents, or they use recent pollen that has been chemically treated to assume a similar state. Melittopalynologists and allergologists generally work with untreated, sometimes stained, grains, although some very important studies in the former field have been carried out with treated grains. The reason for such treatment is that the majority of diagnostic features are found in the exine. The presence of the other layers obscures these features. Untreated grains are unsuitable for analysis of the morphology of the exine.

Palynologists have not been much interested in the interior of the grains, which is still largely unknown. Except for the constituents of the nuclei, the pollen morphology of cytotaxonomists is generally rather poor; their size measurements, carried out on untreated pollen, are subject to great and incalculable errors.

EXINE

The pollen exine, as revealed by the best light microscopes, nearly always consists of two layers which I shall call the "endexine" and the "ektexine". Erdtman, who originally coined these terms, later substituted the terms "nexine" and "sexine". Apparently there is some fundamental difference between the outer and the inner part of the exine, either chemically or structurally. By suitable staining the outer part takes a strong colour, while the inner part remains almost or completely unstained. As this inner

layer is in many cases very thin, one might suspect that the apparent lack of colour could be a simple effect of different thickness. However, sections of, e.g., *Centaurea cyanus* pollen (with a very thick inner layer), show that the inner part of the section remains unstained, while the outer one stains heavily. As the staining mechanism of pollen membranes is unknown, it is premature to decide whether this differential behaviour is due to differences of structure or of chemical composition. The ektexine I define as the part that takes the stain, the endexine as that part which remains unstained.

By contrast, Erdtman (1952, p. 465) defines his present terms in a purely morphologic manner. Nexine is the inner, non-sculptured layer of the exine, while sexine is the outer, sculptured layer. In contrast to the term " sculpturing ", which Fægri-Iversen uses for the surface configuration only, sculpture in Erdtman's sense also includes the elements that carry the surface configuration and provide the material basis for it. From the point of view of formal logic, Erdtman's term is perhaps not unequivocal; in practice Erdtman's sexine is constituted by the columella layer plus the tectum, if the latter is present.

If we define ektexine by means of its staining capacity, it is possible that it does not in all cases coincide with the sexine, but that there may exist staining elements also nearer to the centre of the grain than the bottom end of the columellae, cf. the foot layer (below).

Erdtman has described a few species in which the nexine is very thick and is perforated by narrow channels (tubuli). These very interesting observations stand isolated, and their general significance can not at present be properly evaluated. In the overwhelming majority of all pollen grains hitherto studied, the endexine forms a structureless envelope, generally rather thin in relation to the outer layer. I have not been able to convince myself about the correctness of Erdtman's assertion that the endexine is stratified into an outer, thicker, less refractive layer (ectonexine) and an inner, thinner, more refractive one (endonexine). In the electron-microphotograph of *Corylus* recently published by Erdtman (1955, p. 143) there is no distinct stratification in the nexine. On the other hand, there is a very distinct one in the section of a grain of *Clivia miniata,* published by Afzelius (1955, pl. 1). The

latter electron-micrograph is rather enigmatic, and it is interesting to note that the author herself draws the conclusion that "the most apparent difference in structure is to be found between the endonexine and the rest of the exine" (l.c., p. 142). Perhaps we should have a third designation taking cognizance of this perception!

The ektexine presents a more complicated picture than the endexine. When it is most complete, it is formed of three strata—the foot layer, the columellae,[2] and the tectum. The foot layer is rarely observed, the tectum is frequently missing, but columellae are always present. The exceptions are formed by a few species in which both layers of the exine seem to be rudimentary or completely missing (*Zostera*), or where the very thin exine apparently corresponds to the endexine in normal grains (*Juncus*). In the great majority, however, both strata appear to be present, even though the columellae are sometimes small and few, and are easily lost (*Juniperus*).

In the following I shall deal chiefly with angiosperm pollen. Cryptogam spores and even gymnosperm pollen are organised in a different manner (the chemical composition also is different), and it is hardly possible to-day to give final judgement on parallelisms in the structure of the exines (exospore). With the reservation mentioned, the most important layer of the ektexine is that of the columellae, and the main advances in pollen morphology have been concerned with it. Typically it consists of separate, cylindric elements, "planted" individually on the endexine.

This typical state is modified in different ways. In some grains the lowermost part widens out, forming the above-mentioned foot layer which is very thin and difficult to observe, even in sections. It may be over-looked, especially if staining has not been resorted to, and it is hardly possible to state anything about its frequency. It may be of very rare occurrence, like the tubuli, and it may be more widespread than we know to-day. *Dipsacus* may be quoted as an example: in that grain the foot layer apparently forms a continuous layer on the outside (excentric side) of the endexine.

[2] In 1950 Fægri-Iversen used the term "granules" for "free columellae", i.e., columellae which were not united on top by a tectum. However, Erdtman has shown (1952, p. 463) that our use of that name rests upon a misapprehension, and I have therefore dropped it and shall use the term "columellae" in all cases.

There is also the possibility that what has been described as a thick endexine may in some cases also comprise a foot layer, but at present this is conjectural.

COLUMELLAE

It is important to note that the spaces seen between columellae in the light microscope are real; further, that they are not artifacts produced by treatment during the preparation. Electron micrographs of chemically untreated grains show this (Dahl and Fernández-Morán et al.).

The distance between the individual columellae is highly variable. Evenly distributed columellae of constant size are found in many types of pollen. In *Juniperus* they are scattered at comparatively great distances from each other all over the surface of the grain. In others they are more or less crowded, in extreme cases so much so as to be virtually indistinguishable (Gyrostemonaceae, Erdtman, 1952, p. 198[3]). Another good example of crowding is found in Betulaceae, the upper parts (ca. ¾) of the grains being fused into such a continuous layer that the individual grains are invisible. The lowermost parts are much thinner and are consequently separate from each other; as a matter of fact, they are so thin that in sections they are all but invisible in ordinary light microscopes. However, Erdtman's electron-microphotograph (1955, p. 143) of *Corylus* shows that the interpretation is correct.

The fused layer, formed by the upper parts of the columellae, has been called the "tectum" by Fægri-Iversen, the "tegillum" by Erdtman. It is very unpleasant that the term "tectum" has, since it was originally published, been used by other authors (Erdtman, Potonié) in a different sense, in one case also redundantly.

In cases like Betulaceae the tectum is continuous, apart from possible sub-microscopic perforations. Such a grain is typically tectate. The other alternative is, of course, an intectate grain, where there is no fusion of the upper parts of the columellae. There exists a series of gradations between the two extremes; tecta with small perforations occur, e.g., in some Cariophyllaceae.

[3] In one species, placed at my disposal by Dr. Erdtman, columellae can be distinguished near apertures, but not elsewhere on the grain. It will be described later by Dr. Erdtman.

A very common type is the reticulate, where the tectum forms a reticulum, supported on columellae distributed in the same manmer. If the walls forming the meshes of the reticulum are narrow in relation to the area of the meshes, the grain must still be considered intectate. If they are very broad, the structure via foveolate passes into tectate.

In unstained grains it is often rather difficult to decide whether a grain is tectate or not. Staining with a red stain (fuchsin B or magenta) generally brings out the difference, especially at lower magnifications, the intectate grains taking a more bluish hue. The difference is easily seen, especially if comparison can be made with tectate grains.

From the simple cylindric type of columella, there are stages of complication. In the first stage columellae branch out, tree-like. Further, the branches may anastosmose in varying degrees, ending with the extremely complicated patterns in Compositae-Liguliflorae, where columellae have been dissolved into a three-dimensional network (cf. Mühlethaler, 1953). A different and perhaps even more complicated type occurs in Dipsacaceae where the branches seem to coil, snake-like, forming an irregular tangle in the outer part of the columella layer.

Another complication occurs in the tectum. In its simplest state this is merely an envelope of even thickness, covering the columellae, either at an even distance from the endexine or, if the columellae are of unequal length, forming an exterior pattern, the sculpturing. Such exterior patterns formed underneath a tectum of more or less equal thickness occur, e.g., in the Compositae-Tubuliflorae. In other cases the columella layer is more or less even, while the pattern is formed by a variable thickness of the tectum. Extreme cases are to be observed in the upper parts of the ektexine forming the crotonoid pattern in some Euphorbiaceae.

APERTURES

Most pollen grains are aperturate, the number of apertures varying from one to perhaps as high as about 100. As is well known, they have two distinct functions, namely, as exists for pollen tubes and as harmomegathi, i.e., flexible parts of the otherwise rigid exine, permitting changes of shape and size with varying water content (Wodehouse, 1935, p. 542). The typical harmo-

megathus is a boat-shaped furrow which folds in when the grain is dry, and folds out when the latter expands under moist conditions. The endexine continues in the fold but may be thinner than in the rest of the grain. Ektexinous elements may be present and are then generally smaller and more scattered than in the rest of the grain. In many cases columellae also become smaller and more scattered in the vicinity of the furrow.

The typical exit is a circular pore. In some cases it is closed by an operculum, i.e., a piece of endexine of more or less normal thickness and carrying some ektexinous elements. The operculum is separated from the rest of the exine by a circular zone of very thin endexine. When the pollen contents swell, the operculum is generally pushed off. If there is no operculum, the pore appears as a hole in the exine; most probably an endexinous cover has been lost or torn when the pollen contents penetrated the aperture (cf. Baker, 1955).

Pores occur in the furrows or isolated in exines without furrows. Investigators have been inclined to consider such pores as rudimentary furrows with a remaining pore. If there are furrows only, no pores, the pollen tube generally emerges through the thin endexinous fold in the bottom of the furrow, leaving a more or less irregular hole with tattered edges. In many cases this exist is preformed in some way, and there are transitional stages to the regular pore.

While the association of pore and furrow is generally very close, there are some types in which there is a certain independence, with accessory furrows, extra-colpate pores, diporate furrows, etc. It remains to be decided whether all these cases are homologous with ordinary pores and furrows.

The terminology of apertures is at present rather unsettled. Fægri and Iversen have restricted themselves to the two terms "furrows" (colpi) and "pores", giving them purely morphologic definitions. Erdtman, on the other hand, has preferred a more elaborate nomenclature, in which the position and implicitly the genesis of the apertures are indicated in the name. Such a composite point of view must cause difficulties in practical application; I may refer to Chadefaud's difficulty in deciding whether an aperture should be considered eu-sulcal or pseudo-sulcal, because he could not decide whether it was distal or not—as a matter of fact,

the whole problem is wrongly put: as the aperture was a pore, the question was whether it was eu-ulcal or pseudo-ulcal, if we follow Erdtman's terminology.

ELECTRON MICROSCOPY

When writing above that no major improvement of microscopical technique has taken place since the introduction of the apochromatic lens, I did not include electron optics. While very much remains to be done in pollen morphology with an ordinary light microscope, it is obvious that we must look to the electron microscope for radically new information. Till now, little has been done in palynology by means of the electron microscope. Pollen grains are not very suitable objects, but some very important features of them have been revealed by this instrument. The investigations may be divided into two groups, the first using medium magnification, i.e., magnification of about 5000. Apart from the proof that the spaces between columellae are really empty, as mentioned above, this technique has brought us nothing that is fundamentally new. It has, however, provided sorely needed confirmation of some conclusions based upon the utmost powers of the light microscope, e.g., the columella structure in Liguliflorae. Besides Dahl and Fernández-Morán's pioneer paper, that of Mühlethaler (1953) may be mentioned here. Very much remains to be done in this field of medium magnification, and we may expect extremely important new information from this source.

The other line of investigation, utilising the utmost magnification available, has till now been followed only in the Bromma laboratory, as far as I know. By using the extreme refinements available, int. al., the Sjöstrand microtome, Mrs. Afzelius has already been able to shed very important light on the fine structure of exines and exospores, as some of the main work has been carried out with *Lycopodium* spores. The pictures (Afzelius, Erdtman, and Sjöstrand, 1954) indicate a lamellar structure, the lamellae having a minimum thickness of ca. 130 Å, corresponding to two elementary lamellae (observed by roentgen diffraction) of ca. 60 Å. Very rarely has a lecture to an international congress to be given encore, but that happened (and I think, rightly) when Mrs. Afzelius presented her results to the 1954 Paris Congress. Structural differences are indicated between different layers, but so far the results, acquired with great difficulties, are too few to permit

general conclusions. I may add that Dr. Erdtman has shown me unpublished photographs indicating that the peculiar structure of Dipsacaceae represents some rather knotty problems of its own.

Clearly, a great future is awaiting the study of both external and internal structure by means of electron microscopy, and we may look forward to a series of important contributions in this field, partly revolutionizing our concepts of the exine, and partly, we may hope, confirming our now sometimes rather hazardous conclusions.

NOMENCLATURE [4]

In the preceding chapter I mentioned that the terminology of pollen morphology is still rather unsettled. To an even greater extent the same pertains to the taxonomic nomenclature of pre-Quaternary pollen and spores of unknown relationships. Because of the unsystematic and unpredictable variation of pollen within different taxa,[5] the only grains that, strictly speaking, can be identified to "mother species" and thus be assigned to their proper place and name in the system of plants, are those taken directly from anthers.[6] Even with recent species, specific identification of pollen grains found isolated from their "mother plant" is only rare possible. We have to rest satisfied with genus, tribe or even family, unless phytogeographical or other auxiliary evidence indicate that only one species is present.

Strictly speaking, pollen grains found isolated in pre-Quaternary deposits are unidentifiable to taxon, even though their similarity—especially in Tertiary deposits—is sometimes sufficiently great to warrant tentative inclusion in a modern taxon. The older a deposit is, the greater is the danger that some extinguished taxon has possessed pollen grains similar to those of a living one. The great majority of pollen types found in pre-Quaternary deposits must perforce be given "provisional" names, pending specific identification, which will be achieved for only an extremely small minority.

Provisional names have caused much discussion, and the field is at present in a state of complete confusion. It is perfectly clear

[4] See the article on this theme by Pant in the January, 1954, issue of The Bontanical Review.

[5] A taxon (pl. taxa) is any systematic unit, irrespective of rank.

[6] Even in such cases caution may be necessary: when preparing this article I wanted a pollen preparation of *Asclepias*. I got plenty of *Ambrosia* pollen from anthers of *Asclepias*, but nothing else. Similar cases are known from literature, where they have not always been correctly interpreted.

that pollen grains belong to what the Rules of Botanical Nomenclature call " form-taxa ", which have been in use for more than a century and have had a status under the Rules since 1935. Pollen grains are even expressly defined as such in Appendix I to the Stockholm edition of the Rules.[7]

For the names of form-taxa definite rules have been laid down in the fourth appendix to the Stockholm Rules. The self-evident fact that the nomenclature of fossil pollen grains must follow these rules was also expressly stated at the palynological symposium arranged by Dr. Erdtman in connection with the Stockholm Congress (see Proceedings, pp. 890-891). No official resolution was formulated—it would have been completely redundant. Unfortunately, not everyone dealing with fossil pollen grains is a botanist, and even fewer are versed in the intricacies of the Rules of Nomenclature. The consequence is the present deplorable confusion, to get away from which palynologists are now working hard.

Form-species in pollen grains have been described incidentally for a very long time, but the first to propose, formally and systematically, form-taxa in fossil pollen grains was, as far as I am aware, Potonié in 1931, who divided the material between two form-genera, Sporites and Pollenites. Considering that the chances of finding pollen grains or spores of a plant is at least as great as that of finding any other organ, it is evident that the number of necessary form-taxa will be very high, even though pollen or spores of many species are not sufficiently distinct to be kept apart. Two form-genera are therefore definitely insufficient, and the two-genera stage was passed long ago. As a matter of fact, the two names quoted are at present problems in themselves because they are still with us, having been formally established—but they have no definite meaning to-day. Perhaps we shall have to treat them in the same way as, e.g., the Linnean " Lichen ", to which they correspond. In any case " Pollenites " is not the only cumbersome piece of luggage that palynology has acquired during the journey.

The main question is: How many of the names proposed so far have a real status under the Rules? I presume that in most cases the authors have complied with the general conditions of valid publication. The special rules in force for fossil plants—with application also to form-taxa—are that diagnoses must be accom-

[7] In the following, the Rules are quoted after Lanjouw, 1952. In the relevant articles no material changes were made in Paris, 1954.

panied by an illustration (Art. 45) and that they need not be in Latin (the relevant Art. 44 specifies recent plants). The Amsterdam Congress also requested (Appendix IV, art. 18 bis, b) specific quotation of a nomenclatural type, but this did not get official status. As a matter of fact, a type is not necessary for any taxon published before January 1, 1958 (decision of the Paris Congress, see Taxon 4: 145 and 152). Some of the names published were unaccompanied by any illustration and are consequently invalid, but we must consider most of them as validly published, and shall have to accept them or prove that they are to be referred to synonymy.

There has been much confused talk about natural and artificial, and semi-natural classification. Again, the Rules are quite specific and leave us with two alternatives only:

If a pollen grain can be identified as belonging to a known taxon —living or fossil—no special name need be or can be attached to it. We cannot, under the Rules, have a form-taxa synonymous with a taxonomic unit; it would come in the same category as, e.g., *Sphacelaria* in relation to *Claviceps*. It may be used vernacularly in the laboratory, but it has no status under the Rules. A fossil pollen grain referable to, e.g., *Alnus,* is an *Alnus* pollen grain (if the identification is not quite certain, it may be called a cfr. Alnus pollen grain), and no Alnopollenites or anything like that. Alnopollenites is an excellent name and may be very appropriate for a form-genus (it may even be unavoidable if properly published), but it does not validate the pollen grain as belonging to any member of the genus *Alnus*. We may hope that it indicates a pollen grain similar to those in *Alnus,* but even that is not necessary under the Rules.

If the fossil pollen grain or spore can not be identified with that of any known taxon, it must be referred to an existing form-taxon, or a new one must be established for it. Under the Rules there is no other possibility. Palynological literature is full of Alnopollenites, Quercoidites, and what not, and these names, if they are validly published, must be accepted, but they do not, and they can not, indicate anything more than a morphologic similarity. Natural relationships can not be expressed by such names.

It would be extremely convenient if pollen form-taxon nomenclature could be constructed on a purely descriptive basis exclusively with names like Hexacolpites, Tricolporites and Aletes, con-

forming to a pre-arranged morphologic system. Such names have the great advantage of being non-suggestive of natural relationships. Unfortunately, it is too late, as all the other names are already there, and we can not invalidate practically the whole body of previously published names, however desirable that would be. For the future we can do no more than ask palynologists to follow certain suggestions, but we can not, under the Rules nor in any other way, force people to adopt a specific nomenclature. Perhaps a good example may be the best incentive, and I may refer, e.g., to Klaus' description (1954) of "Sporotrapoidites illingensis" as an excellent one, although perhaps verbally a little too copious. I understand that this example was inspired by Dr. Erdtman.

There is one type of name which seems to be self-invalidating, exemplified by the genus name "Tricolporo" which—in my opinion fortunately—is unacceptable under the Rules. Art. 30 demands that "the name of a genus (should be) a substantive or an adjective used as a substantive in the singular number". As a grammatical phenomenon, "Tricolporo" is beyond my comprehension, and it certainly is not what the Rules demand.

At the Paris Congress in 1954 Potonié presented a proposal which contained a demand for specification of the relevant nomenclatural rules (Lanjouw, 1954, p. 155). In themselves these proposals are excellent, but I feel that they are redundant, since the Rules already contain the necessary provisions. However, we shall have to see what happens to the proposal in the Committee of Paleobotanical Nomenclature, to which it has been referred.

The constant disregard of nomenclatural rules by a majority of palynologists has led to disastrous results, and much work lies ahead before we can again face comparatively smooth sailing. An Index Sporomorpharum, as announced by Erdtman (1954) in the introduction to the first volume of Grana Palynologica, would mean a very great step forward in this work.

POLLEN ANALYSIS

I. Quaternary Deposits

Although fossil pollen and spores were recognised very long ago, even in pre-Quaternary deposits, the modern era of pollen analysis, and the subsequent development of palynology, start with von Post's 1916 lecture on Quaternary deposits. The original pol-

len analyses were technically very simple. Nevertheless, they not only were adequate but they also represented an enormous advance over previous methods in Quaternary geology.

The simple analyses were adequate because they were concerned with comparatively simple problems: the vegetation with which they dealt was extremely favourable, consisting chiefly of forests which were of simple composition, and in which pollination was chiefly by wind. For a first approximation the history of Middle Scandinavian vegetation is the history of these forests. Another circumstance also contributed to the good results: Middle Scandinavia forms the transition between two major vegetation belts, the boreal conifer forest and the mesophytic deciduous forest. Any climatic change is bound to affect the equilibrium between the two and to register dramatic changes in the composition of the forests. Owing to the irregularity of the topography, the transition belt between the two vegetation types is also very irregular, and everywhere secondary equilibria register the changes.

For these reasons the first primitive analyses were adequate. That they also represented a great step forward is evident: for the first time geologists acquired an instrument for direct quantitative registration of the changing equilibrium. Previously, registrations were qualitative and indirect, furnished by more or less accidental finds of macro-fossils.

For the first 15 years pollen analysts were, on the whole, content to work along the classical lines, the area of investigation gradually expanding, especially in Europe north of the Alps, where problems are essentially similar to those in Scandinavia. Investigators were kept busy clearing up the primary problems of forest history, shore-line movements, etc.

However, demands for greater precision and refinement gradually made themselves felt. Even in Middle Scandinavia forests do not dominate exclusively today: "cultivation steppes" occupy great areas. The history of the cultivated part of the landscape presented itself as an urgent problem, one that could not be taken up by the classical methods. von Post himself realized that the problem was there, and had the idea that a relative increase in grass pollen incidence would give the solution. We know to-day that he was both right and wrong: there is an increase of grass pollen, but it is frequently masked by and imitated by other changes in the grass pollen curve, independent of cultivation.

Therefore the grass pollen curves can not be evaluated without auxiliary evidence. In the late thirties the new principles were gradually worked out, chiefly by Firbas and Iversen, culminating in Iversen's study (1941) of the pollen-analytic registration of the first land-clearance.

This has had profound consequences and has opened quite a new way of collaboration between pollen analysis and prehistory. Before man's influence was discovered in the pollen diagrams, archaeological objects could be treated like any other fossil, and dated and crossdated accordingly. Some of these datings have been very important, but mere dating furnishes meagre data for the history of culture. Now, dating has to a great extent been taken over by C^{14} analysis. By the principles forwarded by Iversen in the work mentioned, the influence of prehistoric man on his surroundings can be traced with a wealth of details which sometimes approach the uncanny. Much important work has already been published along these lines, but the most important is still unpublished, such as that of Troels-Smith and his collaborators in Denmark, a work well known to many colleagues who have seen this project in the field. Experience has shown us that agricultural man influenced his habitat profoundly, introducing new species, among both cultivated plants and weeds, and changing the existing equilibria between vegetation types, all of which registers in pollen diagrams.

As a consequence of these new investigations we know to-day that since the introduction of extensive agriculture in Europe at the beginning of the Neolithic period, our landscape, including the forests, is no longer "natural", but is a function both of existing natural conditions and human interference. In other parts of the world, where pollen analyses have been carried out, the agricultural history is generally shorter, and cultivation less intensive than in Europe, so the problems do not present themselves in the same manner. But in all climatic regions, and in all periods, analyses have shown that the introduction of agriculture involved a much greater revolution than was previously supposed.

The problems posed by these new results, which revolutionized the whole concept of vegetational development in Europe, could not fail to have profound repercussions on the whole outlook of pollen analysis technique. This is apparent in two fields, viz., de-

mands for identification of previously unknown pollen types and for greater statistical precision.

The number of forest trees in northern Europe is very small, and "classical" pollen analysis could be carried out by a person knowing a dozen pollen types in all. The number of indicators of field and meadow vegetation, of cultivated plants and weeds, is very great, and adequate analysis presumes that practically all pollen grains are identified. There are many aggregate pollen types which have not yet been cleared up, like Leguminosae or Cruciferae; it will be necessary to find out how many of them can be identified to genus or species. There are other types which will never be cleared up to more than family level, e.g., grasses in general. But to-day we can not rest satisfied with a limited number of standard pollen types: we must try to identify every one in the samples, to find out whether it has an ecologic tale to tell.

Many of these pollen types are so rare that one cannot expect to find sufficient for a curve. They always occur as scattered grains, and even to find these may incur the investigation of an enormous amount of material, never dreamt about by previous workers. The scarcity of such pollen may be due to scarcity of the species in question, but it may also be a result of low pollen production and of an efficient pollination mechanism with small loss of pollen in the transfer. Also, habitat and vegetation layering are important, species of dry ground and of the lower layers of vegetation being at a disadvantage. Such species may be of great diagnostic value, well worth the work inherent in the search for them.

With such plants, even a single grain may be sufficient as an indicator. On the other hand, if a species is a prolific pollen producer and dispenses its pollen freely, the indicator value of single grains is practically nil. This is especially the case if relatively few pollen grains have been counted. One pollen of spruce among 100 grains counted amounts to 1%, which in the case of spruce in a pine forest region is sufficient to indicate sparse local occurrence of the spruce. But if that grain is still single when 10,000 have been counted, there is no doubt that spruce did not occur in the neighbourhood. In order to check the validity of finds like the former, it is necessary to obtain a very great degree of statistical precision, and generally also to follow the fate of the species in question throughout the whole diagram, which means to produce a

curve with the same precision throughout and leading to a known situation, preferably the present. In the early days it was customary to count 150 pollen grains per analysis; to-day some diagrams are published in which as many as 5,000 grains or more have been counted per analysis. By means of such diagrams it is generally possibly to differentiate between locally produced pollen and pollen that has come from a wider territory, and it even has been possible to utilise the long-distance part of the diagram for dating purposes, this component being an integration of the pollen rain of a very wide area. This, however, demands statistical precision much higher than the percentage level, and it also presumes that the general vegetation development of the surroundings is well known.

The transfer of interest from the simple problem of forest development to the more complicated one of development of all vegetation types has led to different arrangements of the so-called basic pollen sum, which forms the basis of percentage calculations. von Post's idea was that this basic sum should include all species which represented areas of their own. In forest analyses this meant that the sum should consist of the pollen of the trees forming the upper canopy only, while the shrubs, including *Corylus,* were excluded. The idea was inherently sound, but *Corylus* is a good example of how badly it could work out at times. Under-story *Corylus* is a very poor producer of pollen, and there is reason to believe that its pollen is not spread very far. The *Corylus* pollen which we find in the regional pollen rain comes mainly from trees flowering in the open, e.g., along forest edges, and representing areas of their own. It is easy to find similar cases in other vegetation types.

But von Post's principle still is valid: the basic pollen sum should represent the totality of the vegetation we are considering. Thus, if we want to analyse forest development only, it is sufficient to make a diagram of classical type, constituted by forest tree pollen only. If we want to analyse what has happened in those areas which were not covered by forest, we make a diagram of non-arboreal pollen, i.e., pollen of grasses, sedges, heathers, etc. To what extent entomogamous plants should be included in the regular sum, is still a matter of controversy. And finally, if we want a picture of the development as a whole, we must include practically everything, trying to keep away from deposits in which the locally produced pollen over-shadows the regional development. The

problems posed by differences in pollen productivity become extremely acute in such cases.

We may, however, also go the opposite way, and make specialised sums to study what happens within a special sector of the vegetation, e.g., the conifer forests, neglecting, for the time being, everything else. Thus, the single basic sum of classical pollen analysis has given way to bases of calculation which vary with the problem. As the problem may vary during a time sequence, it is by no means certain that the basic sum suitable for the oldest part of the deposit is also suitable for the younger. The formation of a basic sum has become a very important part of a pollen-analyst's job, and very much depends on a correct solution of this primary problem.

What has been said about the recent development of pollen analysis above, mainly pertains to North and Middle Europe. Application of pollen analysis outside the classical area poses the problem of the basic sum even more acutely. The definition of a correct one is a conditio sine qua non for successful analysis of vegetation history. Pure forest pollen analysis is of very limited value in a region where the fundamental equilibrium is not between different forest types but between forest and non-forest (tundra or steepe), or even between different non-forest types.

The problems posed by the much richer floras, e.g., of American conifer forests, as compared with the European ones, do not seem to be fully solved as yet. Some of the pollen categories are rather heterogeneous and ecologically ambiguous. In many cases this seems to be unavoidable. It is not possible to arrive at a safe differential diagnosis between the pollen of taxonomically related but ecologically different species. And the array of types in the diagrams is slightly bewildering to a non-American reader. A closer ecologic analysis and comprehension of types to a smaller number of ecologically contrasting groups would be a great help—if feasible.

Some of the most astonishing results of the refined methods of modern pollen analysis have been achieved in the study of the North European late-Glacial. It should be remembered that both the recent and the fossil flora of the area are extremely well known and that ample information has been available, even of its late-Glacial history. It has therefore been an almost stunning surprise to find a whole array of species, a whole floral element, that evi-

dently played a great role then, but which later disappeared more or less completely. *Helianthemum* spp., *Sanguisorba minor, Centaurea cyanus, Ephedra* cfr. *distachya, Pleurospermum austriacum* and others are very rare in or completely absent from North Europe to-day, and we never had a suspicion that they once were important constituents of late-Glacial vegetation. This floral element indicates rather peculiar climatic conditions, but the picture is consistent: comparatively dry climate during the most favourable period (Allerød, 10,000–11,000 years ago), decidedly temperate then, and for the rest hardly so cold as we have been inclined to believe. The frequent occurrence of arctic-alpine species must be explained as a lingering-on effect, due to small competition in open vegetation types. It is also tempting to speculate on the effect of humus and mycorrhizal conditions. Recent investigations indicate that similar conditions prevailed also in North America during the same period (Andersen, 1954).[8]

The preceding discussion has not touched upon one very important aspect, viz., the theoretical foundation of pollen analysis. Pollen analysts have not shown great interest in this topic, and it is significant that the most thorough recent discussion has been published by a botanist who never had any practical contact with pollen analysis (Fagerlind, 1952). After an extremely interesting discussion of the statistical problems posed by irregular occurrences, differences in pollen production, etc., he concludes by laying down a series of restrictive conditions which can practically never be realized in actual pollen-analytic work. He then continues: " If any of the said pre-requisites are lacking, it is very hazardous to draw conclusions from the diagrams ". Ignoramus et ignorabimus. I should like to add, perhaps a little flippantly: unless the investigator happens to be a pollen analyst.

There is no doubt that Fagerlind is right: it is very hazardous to draw conclusions from a pollen diagram, and one shudders at the ease (recklessness may be a better word) with which it is fre-

[8] Considering the present possibilities for close vegetational analysis, and considering the results reached by other pollen-analytic schools, it is remarkable to find, even in Europe, works that with regard to methods and view-points do not represent any advance over the 1916 stage, if they reach that high. It hardly serves any purpose to conceal that papers like the last major one issued from the Strassbourg school of pollen-analysis, do not reflect favorably on the school from which they originate, and that methods should be radically changed if pollen analyses are also in the future to be carried out in Strassbourg.

quently done. The inherent problems do not lend themselves to mathematical calculation, and all experience has shown that if pollen problems are treated solely from a formal mathematical point of view, we automatically arrive at the same pessimistic conclusion as did Fagerlind. The remedies against the inherent pitfalls of the pollen-analytic method are simple: caution, intimate knowledge of the ecology of the plants in question, and of the ecologic conditions of the area under investigation. Especially the latter is extremely important: under different ecologic conditions the same pollen-analytic phenomenon may mean opposite things. If pollen diagrams are treated as problems of ecologic botany, but neither as mathematical curves nor as indicator fossils, the possibilities of valid conclusions are still there, as they were before Fagerlind issued his very valuable warning. Indeed, intimate knowledge of the ecologic situation, together with knowledge of the general pollen-analytic development within the region, permits us to draw conclusions, even from those features of the diagram which were previously considered as grave sources of error—like over-representation from local vegetation, or long-distance pollen transport (example in Fægri, 1954).

Historically, pollen analysis developed from the peat-bog investigations of the first decennia of our century. Pollen analysts therefore also collected material from peat-bogs. Experience has, however, shown that lake sediments are more favourable because sedimentation in lakes is generally more regular, and the local pollen production is both smaller and easier to recognise. The locally produced pollen can therefore be deducted from the total. In those early days of pollen analysis much emphasis was laid on the representativity of a sample: the ideal was to collect samples from the middle of a great bog, or a lake, far away from the shores with their aberrant vegetation zones which might influence the diagram unduly.

This is an excellent principle which still holds true if our object is to get as pure a regional registration as possible. However, special problems have forced us to utilise material which does not fulfill these demands, and we have learned a great deal about how such material can be utilised, and how its weaknesses can be remedied. Analyses from such unsuitable deposits give diagrams which certainly are subject to local influence, but, provided ecological conditions are known, the local influence can be evaluated.

The extreme is reached in raw humus analyses. At any rate some kinds of raw humus preserve pollen exines exceptionally well, while practically everything else has been reduced to " humic acids ". Almost pure pollen suspensions can be obtained simply by boiling samples in 10% KOH. Under favorable conditions such accumulations may become very old; I have even found a case of apparently continuous accumulation of raw humus during the last 7,000 years or so. The pollen diagrams obtained from such deposits are naturally subject to extreme local influence and reflect conditions within a radius of 50 or 100 metres, perhaps a little more if there has been no forest. At any rate, what we do get is a registration of the development of an individual plant community, and, while it may be doubtful that general climatic inferences can be drawn from such a diagram, it opens up new vistas, and we perceive the possibility of establishing palaeophytosociology as something like an objective science (Fægri, 1954).

We may summarise the trends apparent in modern Quaternary pollen analysis as follows:

a) A higher degree of statistical probability by the use of a greater number of grains per analysis in order to increase the accuracy of conclusions.

b) More acute morphological analysis.

c) As a consequence of this we get more definite specific diagnoses.

d) The more acute specific analysis gives greater possibilities for ecologic conclusions.

Thus we may state that modern pollen analysis is moving away from the chronologic problems that were so important in the early days, and towards ecologic problems. That again means that pollen analysis to-day is not a technique for geologists, but for botanists; it is not a technique for general geologic research, but for palaeobotanical investigation; and only a trained field botanist will be able to utilise it fully.

II. Pre-Quaternary Deposits

Problems in pre-Quaternary analysis are different from those dealt with in the preceding chapter. Even with the most acute morphologic description, specific identifications are in most cases unobtainable, and, even though it should be possible by some

means to attach a specific name to a pollen grain or a spore, ecologic inference remains as remote as ever. As a matter of fact, for ecologic inference any artificial form-species name may be in most cases just as suitable. All such inference will be indirect, e.g., from the nature of the deposit, and its further use exposes the investigator to some rather vicious circles of thought. Considering the uncertainty of ecologic inference, statistical refinements are rather redundant for that specific purpose. But in pre-Quaternary analysis another problem enters, which is generally of subordinate significance, especially in late-Quaternary deposits: that of stratigraphical connections.

Pre-Quaternary pollen and spore analysis is still mainly in a descriptive phase, the problems of describing, defining, naming and identifying exines being subjects of major attention. The goal is stratigraphic analysis. In this connection it must be kept in mind that the motives in pre-Quaternary analysis are utilitarian to a much greater degree than in Quaternary. Pollen and spore analysis has proved a very efficient tool for recognition and identification of strata, often on a microstratigraphical basis, from one locality to another. The obvious use has been in coal-fields where pollen analyses have established themselves as indispensable for the scientific evaluation of the fields. Oil prospecting companies have also begun to utilise pollen and spore analysis where other methods have failed or needed corroboration, and there are reasons to suppose that the technique will be no less important in this connection. Unfortunately, the great commercial companies have a tendency to keep both methods and results secret, and very little has been published so far. Apparently the technique has been used for a longer time in the USSR than in the West, and more has been published about it there.

Attempts have been made to utilise palynological records for palaeoecological information. So far, these attempts have been rather isolated, and for reasons given above, they suffer from inherent logical weaknesses. No doubt, however, there is a store of information to be tapped, but with great caution.

The first fossil pollen grains to be described were from a pre-Quaternary deposit. Nevertheless, pre-Quaternary pollen and spore analysis is still in its infancy because it was taken up systematically much later than Quaternary analysis, because in the past fewer have been working in this field, and because the prob-

lems are much more difficult. Even so, pollen and spore analysis is an important method of investigation of pre-Quaternary deposits, and it is certainly one about which we shall hear more in the future. Before the great advances can be made, the present muddle in nomenclature must, however, be cleared up.

PALYNOLOGY AND TAXONOMY

When new species are described, or taxa revised, pollen grains are very rarely considered, and in most of the exceptions palynological description is superficial, to say the least. Consequently, enough palynological data have not been available for inclusion in ordinary taxonomic thinking. This certainly also pertains to other microscopical characters, but pollen grains differ from most of the others in their much greater diversity. Therefore they might be utilised taxonomically to a much greater extent, if data were available.

The appearance of Erdtman's 1952 text-book, referred to previously, represents a turning-point in this respect. By it a wealth of new material has been placed in the hands of taxonomists, and for the first time a palynological survey has been given to the whole angiosperm world. Admittedly, much remains to be done, and even though the survey were complete, pollen morphology is not the only solution of all difficult taxonomic and phylogenetic problems; on the other hand, it should not be neglected. In dubious cases pollen morphology may give decisive indications in one or another way. Palynological characters have the advantage of being rather conservative. They are modified under the influence of different methods of pollination, but these influences have been defined by Wodehouse and do not interfere with the taxonomical value of the characters.

Besides Erdtman's all-embracing work, less comprehensive studies have been published with a purely phylogenetic view-point. Kouprianova (1954) has taken up the whole group of monocotyledons. Her main conclusions are: *a*) palynologically the Helobiae have no connection at all with the rest of the monocotyledons —they must be considered specialised Polycarpicae of Ranunculacean affinity; *b*) Araceae and Lemnaceae form a unit of their own, though undoubtedly connected with the other monocotyledons; *c*) the rest may be divided into two closely connected

branches, which have their origin in, respectively, Palmae and Liliaceae. The former branch, besides Pandanales, contains the grasses and groups leading up to them (Restionaceae, etc.). All the other families go with the Liliaceae: a special group is formed by Juncaceae and Cyperaceae. While perfectly consistent palynologically, the arrangement of some taxa, especially in the Palmae branch, may seem odd, and final judgment must be postponed.

M. van Campo-Duplan has (1950–53) undertaken a great work on the pollen grains of conifers, of which she has described a very great number belonging to the three main families. Naturally, the phylogenetic conclusions from a study within such narrow groups as individual families are more difficult to evaluate for non-specialists, and I shall not enter upon them here.

In many cases the pollen type of a taxon is both constant (stenopalynous) and characteristic, perhaps exclusive. In other perfectly natural taxa, pollen types vary more or less unpredictably, or the type or types may not be sufficiently distinct to be kept apart from those of other, unrelated taxa. If a pollen grain like those dealt with in the first case is found in a plant of doubtful affinity, it may furnish very important indications of the taxonomic position; Erdtman (1954) has given a few examples of this. It is hoped that there will be a " recent trend " in taxonomy toward giving palynological description together with the other morphologic analyses of species to be described, de novo or in revisions. Only, it must be kept in mind that a valid palynologic analysis presumes certain qualifications, without which its value will certainly be very small.

REFERENCES

AFZELIUS, B. M. 1955. On the fine structure of the pollen wall in *Clivia miniata*. Bot. Not. **1955**: 141.

————, G. ERDTMAN and F. S. SJÖSTRAND. 1954. On the fine structure of the outer part of the spore wall of *Lycopodium clavatum* as revealed by the electron microscope. Svensk Bot. Tidskr. **48**: 155.

ANDERSEN, S. TH. 1954. A late-Glacial pollen diagram from southern Michigan, U. S. A. Danmarks Geol. Unders. 2. rk. **80**: 140.

BAKER, H. G. 1955. Aperture membranes in studies of pollen morphology and taxonomy. New Phytol. **54**: 350.

BAKKER, E. M. VAN ZINDEREN. 1953. South African pollen grains and spores. I. 88 pp.

VAN CAMPO-DUPLAN, M. 1950. Recherches sur la phylogénie des Abietinées d'après leurs grains de pollen. 183 pp.

————. 1951. Recherches sur la phylogénie des Taxodiacées d'après leurs grains de pollen. Trav. Lab. Forest. Toulouse. 2, vol. 4, art. 2.

————. 1953. Recherches sur la phylogénie des Cupressacées d'après leurs grains de pollen. *Ibid.* art. 3.

CHADEFAUD, M. 1955. Rémarques sur quelques pollens de plantes tropicales, particulièrement interéssantes des points de vue palynologique ou systématique. Rev. Gen. Bot. **62:** 641.

CRANWELL, L. M. 1952. New Zealand pollen studies. The monocotyledons. Bull. Auckland Inst. Mus. 3.

ERDTMAN, G. 1927–1955. Literature on pollen-statistics published before 1927. Geol. För. Stockholm Förh. **49:** 196. [Repeated biannually or annually in the same journal, under slightly varying titles until the latest: Literature on palynology. XVIII. *Ibid.* **77:** 71].

————. 1943. An introduction to pollen analysis. 239 pp.

————. 1952. Pollen morphology and plant taxonomy. Angiosperms. 539 pp.

————. 1954. Pollen morphology and plant taxonomy in some African plants. Webbia **11:** 1. [Also in Grana palynologica 1, nr. 5].

————. 1955. Från palynologiens arbetsfält. Statens Naturv. Forsk. r. Årsb. **8**(1953–54): 139.

FAGERLIND, F. 1952. The real signification of pollen diagrams. Bot. Not. **1952:** 185.

FERNÁNDEZ-MORÁN, H., and A. O. DAHL. 1952. Electron microscopy of ultra-thin frozen sections of pollen grains. Science **116:** 465.

FISCHER, H. 1890. Beiträge zur vergleichenden Morphologie der Pollenkörner. 72 pp.

FÆGRI, K. 1954. On age and origin of the beech forest (*Fagus silvatica* L.) at Lygrefjorden, near Bergen (Norway). Danmarks Geol. Unders. 2. rk. 80: 230.

———— and J. IVERSEN. 1950. Text-book of modern pollen analysis. 168 pp.

IVERSEN, J. 1941. Landnam i Danmarks stenalder. Danmarks Geol. Unders. 2. rk. 66.

KLAUS, W. 1954. Bau und Form von *Sporotrapoidites illingensis* n. gen. et sp. sporomorpharum. Bot. Not. **1954:** 114. [Also in Grana palynologica 1, nr. 11].

KNOX, E. M. 1950. The spores of *Lycopodium, Phylloglossum, Selaginella,* and *Isoetes.* Trans. & Proc. Bot. Soc. Edinburgh 35: 211.

KOUPRIANOVA, L. A. 1954. Sur la phylogénie des monocotyledones. Essais de botanique. I. Acad. Sci. U.R.S.S., Sér. Bot., U.R.S.S.: 120. [Also bulletin (Trudy) from the Komarow institute 1948].

LANJOUW, J. [editor]. 1952. International code of botanical nomenclature. Regnum vegetabile 3.

————. 1954. Recueil synoptique des propositions concernant la code international de la nomenclature botanique. *Ibid.* 4.

MÜHLETHALER, K. 1953. Untersuchungen über die Struktur der Pollenmembran. Mikroskopie **8:** 103.

PANT, D. D. 1954. Suggestions for the classification and nomenclature of fossil spores and pollen grains. Bot. Rev. **20:** 33.

VON POST, L. 1918. Skogsträdpollen i sydsvenska torvmosselagerföljder. Forh. v. 16. skandinaviske naturforskermøte **1914:** 433.

POTONIÉ, R. 1931. Zur Mikroskopie der Braunkohlen. Tertiäre Blütenstaubformen. Zeits. Braunkohle **1931: 325.**

SELLING, O. H. 1946–48. Studies in Hawaiian pollen statistics. I–III. Bishop Mus. Spec. Publ. 37–39.

WODEHOUSE, R. P. 1935. Pollen grains. 574 pp.

ZANDER, E. 1935–49. Pollengestaltung und Herkunftbestimmung bei Blütenhonig. Vols. I–IV.

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